THE REDUCTION OF TOLERANCE TO THE ANTICONVULSANT EFFECTS OF ANTIEPILEPTIC DRUGS

by

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ABSTRACT

Tolerance has been shown to develop to the anticonvulsant effects of many different antiepileptic drugs. In some cases, tolerance to antiepileptic drug effects is so complete that the drugs become clinically ineffective, even at toxic doses. This is clearly problematic for the treatment of epilepsy. The purpose of this thesis was to identify and investigate factors that reduce tolerance to anticonvulsant drugs. Accordingly, Experiments 1 and 2 were designed to demonstrate potential strategies for preventing the development of tolerance to anticonvulsant drug effects, and Experiment 3 was designed to determine the time course of the effect of convulsive stimulations on the dissipation of tolerance to anticonvulsant drug effects. The recently proposed drug-effect theory of tolerance provided the theoretical framework for these experiments.

The purpose of Experiment 1 was to assess the effect of dose on the development of tolerance to pentobarbital. During the tolerance-development phase, amygdala-kindled rats received one of the following before each bidaily (one every 48 hr) stimulation: either the same high dose (50.0 mg/kg), the same low dose (10.0 mg/kg), a series of doses that increased progressively from 10.0 to 30.0 mg/kg, or the saline vehicle. The rats that received the ascending-dose regimen became significantly tolerant to the anticonvulsant effects of pentobarbital. In contrast, tolerance to the ataxic effects of pentobarbital was significantly greater in the rats that received the high-dose regimen.

The purpose of Experiment 2 was to test the generality of the results of Experiment 1 by assessing the effect of dose on the development of tolerance to the anticonvulsant effects of diazepam. As in Experiment 1, amygdala-kindled rats were exposed to either a high-dose regimen (10.0 mg/kg), a low-dose regimen (1.0 mg/kg), an ascending-dose regimen (from 1.0 to 3.0 mg/kg), or the saline vehicle during the tolerance-development phase. Again, the rats that received the ascending-dose regimen became significantly tolerant to the anticonvulsant effects, whereas those in the other groups did not. In addition, both the rats that received the ascending-dose regimen and
the rats that received the high-dose regimen exhibited a withdrawal effect after the cessation of diazepam injections. The results of Experiments 1 and 2 suggest that the common clinical practice of starting epileptic patients off on a low dose of anticonvulsant medication and then gradually increasing the dose should be re-evaluated.

In Experiment 3, amygdala-kindled rats were first made tolerant to the anticonvulsant effects of bidaily injections of diazepam (2.5 mg/kg). This tolerance did not dissipate in rats that received no diazepam over either a 4-day, 8-day, or 16-day retention interval if they were not stimulated during this interval. In contrast, tolerance dissipated in rats that received bidaily stimulations during the retention interval, even if they received diazepam injections after each stimulation. These results confirm previous findings that the occurrence of convulsive stimulations in the absence of the anticonvulsant drug effect is a key factor in the dissipation of tolerance to anticonvulsant drug effects, and they provide the first systematic evidence concerning the time course of the dissipation—tolerance declined gradually over the entire 16-day retention period.

The results of these experiments make four important points. First, Experiments 1 and 2 provided evidence that commonly employed ascending-dose regimens facilitate the development of tolerance to anticonvulsant drugs. Second, Experiment 1 demonstrated that the effect of drug dose on tolerance development can be different for different effects of the same drug. Third, Experiment 3 confirmed that the cessation of drug administration alone does not cause the dissipation of tolerance to anticonvulsant drug effects. And fourth, all three experiments provided general support for the drug-effect theory of tolerance: the theory that functional drug tolerance is an adaptation to the effects of the drug on neural activity, rather than to drug exposure per se.
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GENERAL INTRODUCTION

Epilepsy is a chronic disorder that is characterized by spontaneously recurring seizures. Approximately 50,000,000 people worldwide are currently suffering from epilepsy, at least 25% of whom have seizures that are resistant to anticonvulsant medication (Porter & Rogawski, 1992). A major problem in the treatment of epilepsy is the development of tolerance to the anticonvulsant effects of antiepileptic drugs; tolerance has been shown to develop to the anticonvulsant effects of most commonly employed antiepileptic drugs (Frey, 1987). In some cases, tolerance to antiepileptic drug effects is so complete that the drugs become totally ineffective even at toxic doses (Browne, 1982). Because the vast majority of epileptics are dependent on medication for the control of their seizures, the development of tolerance to antiepileptic drugs constitutes a medical problem of major proportions (Porter, 1986).

The general purpose of the present experiments was to identify and investigate factors that can reduce tolerance to anticonvulsant drug effects. The theoretical framework for these experiments was provided by the recently proposed drug-effect theory of tolerance (i.e., Pinel & Mana, 1986). Accordingly, the first six sections of the General Introduction deal in sequence with the following topics: (1) the phenomenon of drug tolerance, (2) the implicit drug-exposure theory of tolerance, (3) the drug-effect theory of tolerance, (4) the evidence for the drug-effect theory, (5) the kindled-convulsion model for studying drug tolerance, and (6) the experimental and clinical evidence for tolerance to anticonvulsant drug effects. The general rationale and purpose of the thesis are presented in the seventh, and final, section.
(1) The Phenomenon of Drug Tolerance

Drug tolerance is a decrease in the effect of a drug that occurs as the result of previous exposure to the drug. It is often operationally defined as a shift in the dose-response curve to the right; higher doses of a drug are necessary to produce the same effect, and the same doses produce less effect. However, in other cases there is a flattening of the dose-response curve; there is a decrease in the maximal drug effect, with little change in the effectiveness of low doses.

By convention, drug tolerance is divided into two categories: dispositional tolerance and functional tolerance. Dispositional tolerance is presumed to result from an alteration of the pharmacokinetics of the drug such that the same dose produces either a lower concentration at its site of action (Kalant, LeBlanc, & Gibbons, 1971) or a decrease in the amount of time that the drug remains at its site of action (Lê & Khanna, 1989). This may result from changes in the absorption, distribution, metabolism, or excretion of the drug (see Mana, 1990). Functional tolerance, on the other hand, results from adaptational processes in the nervous system; there is a decreased sensitivity of the neural circuits that are affected by the drug, and as a result the drug no longer produces as great an effect. Functional changes may occur at the primary site of action of the drug or in neuronal pathways downstream from this site (Pratt, 1991).

Many mechanisms have been proposed to account for the development of functional drug tolerance. These include changes in the synthesis, release, or reuptake of various neurotransmitters (e.g., Löschner, 1986; Melchoir & Tabakoff, 1981), neuromodulators (Vollicier & Ullman, 1985), or hormones (Tabakoff & Yanai, 1979; Wood, 1977); changes in cell membrane composition (Goldstein, 1983); changes in the activity of second messengers (e.g., Siggins, 1979); changes in ion conductances across cell membranes (Ross, Garrett, & Cardenas, 1979); and changes in the sensitivity or number of receptors (e.g., Gallager & Gonsalves, 1988). In recent years, research on the mechanisms of functional drug tolerance has focused on receptor changes. Two important
findings have been responsible for this focus (Pratt, 1991). The first was the finding that chronic exposure to a receptor's agonists can decrease the number of receptors (down-regulation) and that chronic exposure to a receptor's antagonists can increase the number of receptors (up-regulation)--for a review see Poste & Crooke (1985). The second was the finding that chronic drug administration can result in a functional change in its receptors; for example, the reduced response to GABA following the development of tolerance to benzodiazepines is thought to be due to an uncoupling of GABA receptors and benzodiazepine-recognition sites of the GABA receptor complex (Gallager, Marley, & Hernandez, 1991; Teitz, Chiu, & Rosenberg, 1989).

Drug tolerance is one of the most widely studied pharmacologic phenomena (see Goudie & Emmett-Oglesby, 1989). Most research on drug tolerance falls into one of four categories: (1) experiments that have been conducted to determine whether or not tolerance develops to particular drugs (e.g., Kalant & Khanna, 1990); (2) experiments that have assessed the effect of various drug-related variables on the development of tolerance (e.g., Kalant & Khanna, 1990); (3) experiments that have examined the relation between tolerance, dependence, and addiction (e.g., Haefly, 1986; Kalant, 1985; Siegel & MacRae, 1984); and (4) experiments that have been conducted in order to derive general principles of biological adaptation (e.g., Kalant & Khanna, 1990). However, despite this massive research effort, our understanding of the mechanisms underlying tolerance and the factors that influence it remains at an elementary level. This lack of understanding is illustrated by the fact that there are still no clear criteria for classifying a particular instance of tolerance as either dispositional or functional. There are four main difficulties in this respect. First, each instance of tolerance is not necessarily dispositional or functional; both types of change may contribute to a particular instance of tolerance (Kalant et al., 1971). Second, any dispositional or functional change that is identified may not be causally related to a given manifestation of tolerance (Demellweek & Goudie, 1983a). Third, the inability to detect a dispositional change does not by default imply a
functional change (e.g., Dews, 1978). And finally, the dispositional or functional change to which a given instance of tolerance is attributed is often too small to account for the decrease in responsiveness to the drug (e.g., Seevers & Deneau, 1963).

Arguably, the major puzzle of drug tolerance is to understand why and how tolerance develops to some effects of a drug while at the same time in the same subject not developing to others, or developing to them at a different rate. In some cases, tolerance develops to one effect of a drug while sensitization develops to one of its other effects; for example, Kuczenski and Leith (1981) showed that the chronic administration of amphetamine resulted in both sensitization to amphetamine-induced stereotypy and tolerance to the effect of amphetamine on intracranial self-stimulation.

(2) The Implicit Drug-Exposure Theory of Tolerance

It has been implicitly assumed that the dispositional and functional changes that underlie the development of tolerance result from exposure of an organism to the drug in question; drug exposure has been assumed to be both necessary and sufficient for drug tolerance. This implicit view has been termed the drug-exposure theory of tolerance (Pinel & Mana, 1986). Because of general acceptance of the drug-exposure view of tolerance, the majority of research on drug tolerance has focused on pharmacologic factors. The following factors have been extensively investigated: the drug dose (e.g., Lê, Khanna, & Kalant, 1984), the duration of drug exposure (e.g., Lê, Kalant, & Khanna, 1986), the interinjection interval (e.g., Giknis & Damjanov, 1984), the half life of the drug (e.g., Okamoto, 1984), and the metabolic characteristics of the subjects receiving the drug (e.g., Kalant et al., 1971).

In recent years, the shortcomings of the drug-exposure view of tolerance have become apparent as evidence has accumulated that nonpharmacologic factors play an important role in the development of tolerance (see Poulos & Cappell, 1991). This evidence has been of two types: evidence that the environments in which drugs are
administered have a major effect on drug tolerance and evidence that what subjects do while they are drugged has a major effect on drug tolerance. Two theories of tolerance have been proposed to account for these findings: the conditioned tolerance theory (e.g., Siegel, 1977; 1986) and the drug-effect theory (e.g., Pinel & Mana, 1986).

The conditioned tolerance theory was developed to account for the fact that subjects often display considerable tolerance to the effects of a drug only if the tolerance test occurs in the environment in which its effects have been previously experienced. According to the version of the conditioned tolerance theory that has been championed by Siegel and his colleagues (e.g., Siegel, 1975; 1989; Siegel & MacRae, 1984), drug-predictive cues function as conditional stimuli that elicit conditional responses, called conditioned compensatory responses. These conditioned compensatory responses counteract the effects of the drug, and each time that the drug is administered in the same environment, the conditioned compensatory responses become greater, and thus more effective in blocking the drug effects. As a result, the tolerance is specific to the situations in which the drug effects have previously been experienced. Although Siegel's Pavlovian theory is the most widely accepted view of situation-specific tolerance, it has also been attributed to the conditional habituation of drug effects (Baker & Tiffany, 1985; Solomon, 1977; Wagner, 1978; 1981), to a combination of conditioning and habituation (Paletta & Wagner, 1986), and to homeostatic responses (Poulos & Cappell, 1991).

The drug-effect theory of tolerance was proposed to account for the fact that what subjects do when they are under the influence of a drug can have a substantial effect on tolerance development. The drug-effect theory provides the framework for this thesis; it is the focus of the next three sections.
(3) The Drug-Effect Theory of Tolerance

According to the drug-effect theory of tolerance, drug tolerance develops to drug effects rather than to drug exposure per se; tolerance is viewed as an adaptive response to the disruptive effect of drugs on ongoing patterns of neural activity. This is a departure from the traditional drug-exposure view; it assumes that drug exposure is necessary but not sufficient for the development of tolerance. The major prediction of the drug-effect theory is that tolerance will not develop to a given drug effect unless that effect is manifested during the period of drug exposure.

The drug-effect theory of drug tolerance can be illustrated with reference to other, better understood, forms of neural adaptation; for example, the adaptation that occurs to the disruptive effects of visual displacement on visuomotor coordination (cf. Pinel & Mana, 1986; Poulos & Hinson, 1984). When a subject first wears displacing prisms that shift his or her visual world a few degrees to one side, visuomotor coordination is severely disrupted. But after some experience with the prisms, the subject adapts (e.g. becomes tolerant) to the visual displacement, and visuomotor coordination returns to normal. What is the factor that leads to this adaptation? Is it the exposure to displaced vision (a theory analogous to the drug-exposure theory of tolerance), or is it the experience of the effects of the disruption of visuomotor coordination on performance of tasks (a theory analogous to the drug-effect theory of tolerance)? The evidence overwhelmingly supports the latter view. Little visuomotor adaptation develops to the effects of displacing prisms in subjects that do not perform visuomotor tasks while wearing them: It is the experience of the disruptive effects of the prisms on the performance of visuomotor tasks that is critical for the adaptation to occur (Held, 1972; Rock & Harris, 1972). In the same way, the drug-effect theory asserts that it is the experience of a drug's effects that is critical for the development of drug tolerance.

For most drug effects, the distinction between the drug-exposure theory and the drug-effect theory is academic, because most drug effects are under normal circumstances
inevitable consequences of drug exposure. For example, a mobile rat injected with an ataxia-producing drug, such as pentobarbital, will inevitably experience pentobarbital's effect on motor activity. In these situations, the drug-exposure theory and the drug-effect theory both make the same predictions about the development of tolerance. However, there are drug effects that occur only if the subject engages in a particular response (the criterion response) while under the influence of the drug. In these cases, experiments can be conducted to determine whether it is the drug exposure or the drug effect that is the critical factor in tolerance development. The drug-effect theory predicts that the development of tolerance to a particular effect of a drug will be contingent on the repeated manifestation of that effect during periods of drug exposure, and that drug exposure alone will not lead to tolerance.

(4) Evidence for the Drug-Effect Theory

Support for the drug-effect theory has been provided by reports of contingent drug tolerance. The term contingent tolerance was first used by Carlton and Wolgin (1971) to describe their observation that the development of tolerance to the anorexigenic effect of d-amphetamine was contingent upon subjects getting an opportunity to eat during each period of drug exposure. Subsequent demonstrations of contingent tolerance have typically used a before-and-after design (Chen, 1968). In this design, subjects in one group receive the drug before each behavioral trial, whereas those in another group receive the drug after each trial. Thus, on each trial, subjects in the drug-before group experience the drug's effect on the criterion behavior, whereas those in the drug-after group do not. Because the subjects of both groups receive the same amount of drug administered in the same fashion on the same schedule, any difference in the development of tolerance between the two groups reflects the difference in the contingency between the drug exposure and the test response.
Numerous examples of contingent drug tolerance have been reported—see Wolgin (1989) for a recent review. For example, contingent tolerance has been demonstrated to the effects of ethanol on maze running (LeBlanc, Gibbons, & Kalant, 1973), on the decay of posttetanic potentiation in the Aplysia abdominal ganglion (Traynor, Schlapfer, & Barondes, 1980), and on male sexual behavior (Pinel, Pfaus, & Christensen, 1991); and it has been demonstrated to the anorectic effects of amphetamine (Demellweek & Goudie, 1983b), cocaine (e.g., Woolverton, Kandel, & Schuster, 1978), and quipazine (e.g., Rowland & Carlton, 1983).

The strongest support for the drug-effect theory comes from a series of studies of contingent tolerance to the effects of anticonvulsant drugs on kindled convulsions. The advantage of using the kindled-convulsion model of drug tolerance to study the effect of the response contingency on tolerance development is that the experimenter can exert complete control over the timing of the criterion response (i.e., the convulsion). This control is not possible with other drug effects such as ataxia, because for these drug effects a subject will experience the criterion response (i.e., ataxia) continuously after the administration the drug. The kindled-convulsion model of drug tolerance was employed in all three of the experiments that compose this thesis.

(5) The Kindled-Convulsion Model of Drug Tolerance

Kindling is a phenomenon in which periodic administration of initially subconvulsive stimulations to certain brain structures results in the development and progressive intensification of elicited motor seizures (Goddard, McIntyre, & Leech, 1969). For example, an initial stimulation of the rat amygdala at an intensity sufficient to evoke afterdischarges elicits little or no behavioural response; however, with each subsequent stimulation, the afterdischarges generalize further from the site of stimulation, and motor seizures begin to accompany them. Then, with each subsequent stimulation, these motor seizures increase in severity. After about 15 daily stimulations, each rat
responds reliably to each stimulation with a generalized clonic motor seizure, which is characterized in sequence by jaw clonus, head clonus, forelimb clonus, rearing, and falling (McNamara, 1988; Racine, 1972). Kindling is permanent (Silver, Shin, & McNamara, 1991); kindled rats that have been left unstimulated for many months respond to subsequent stimulation with a generalized convulsion. Kindling has been demonstrated with a wide variety of convulsive agents, and with electrical stimulation to many brain sites in a wide variety of species (Pinel & Van Oot, 1978; Racine, 1978); however, the majority of kindling experiments have involved electrical stimulation of the amygdala in rats (Racine & Burnham, 1984), because the amygdala had proven to be particularly easy to kindle.

Kindled convulsions are particularly useful for the study of tolerance to anticonvulsant drug effects (see Kim, 1989; Mana, 1990; Rosenberg, Teitz, & Chiu, 1989). Prior to the development of the kindled-convulsion model of drug tolerance by Pinel and his colleagues (Pinel & Rovner, 1978), experiments investigating anticonvulsant drug effects employed convulsions induced by maximal electroshock (MES) or pentylenetetrazol (PTZ). However, both MES and PTZ both induce convulsions that are variable in form and duration, are difficult to measure, and are often associated with subject injury or fatality (e.g., Swinyard, 1980; Voskuyl, Meinardi, & Postel-Westra, 1986). In contrast, kindled convulsions produce little subject attrition and vary little in form or duration, either from subject to subject or from one convulsion to the next in the same subject (Pinel, Phillips, & MacNeil, 1973).

Kindled convulsions are also useful for studying anticonvulsant drug effects because they can act as a predictive model of human complex partial seizures. Complex partial seizures are common; over 25% of all epileptic attacks fall into this category (Pedley, 1992). They are also the most drug-resistant of all types of epileptic seizures, making the control of these seizures a major problem. Limbic-kindled animals are currently the best predictive model of human complex partial seizures (Albright &
Burnham, 1980; Löscher, Jackel, & Czuczwar, 1986; Löscher & Schmidt, 1988); despite the fact that kindled convulsions and human complex partial seizures do not look alike, drug effects on limbic-kindled convulsions in rats are predictive of drug effects on complex partial attacks in humans (Racine & Burnham, 1984; Löscher et al., 1986).

In the version of the kindled-convulsion model of drug tolerance that has been developed by Pinel and his colleagues, rats are first kindled by 45 amygdalar stimulations administered over 3 weeks. Then, they receive a series of bidaily (one every 2 days) stimulations to establish the predrug baseline. Finally, the anticonvulsant drug under investigation is administered prior to each of a series of bidaily stimulations so that the decreasing ability of the drug to block the convulsions can be measured. The severity of each convulsion is measured in two ways. The class of the convulsion is rated according to Pinel and Rovner's (1978) 8-class extension of Racine's (1972) widely used 5-class scale (class 1: head nodding only; class 2: head nodding and jaw clonus; class 3: head nodding, jaw clonus, and unilateral forelimb clonus; class 4: head nodding, jaw clonus, bilateral forelimb clonus, and rearing; class 5: all of the above plus falling once; class 6: a class 5 with multiple falling episodes; class 7: any of the aforementioned symptoms with running fits; class 8: any of the aforementioned symptoms with periods of tonus). The duration of forelimb clonus associated with each convulsion is also recorded. In the three experiments in this thesis, the primary dependent measure was the duration of forelimb clonus, although convulsion class was also recorded. Forelimb clonus duration has been shown to be a reliable measure of seizure severity and to be sensitive to a variety of drug manipulations (e.g., Pinel, Kim, Paul, & Mana, 1989). Forelimb clonus duration and motor seizure class are highly correlated (Pinel, Mana, & Renfrey, 1985), but forelimb clonus duration has the advantage of being a continuous ratio scale. The kindling protocol is described in detail in the General Methods section.

The kindled-convulsion model has in recent years been widely employed in the study of tolerance to anticonvulsant drug effects; its major application has been in the
study of contingent tolerance to anticonvulsant drug effects. Using the kindled-convulsion model and the before-and-after design, tolerance to the anticonvulsant effects of ethanol (Pinel, Colbourne, Sigalet, & Renfrey, 1983; Pinel et al., 1985), pentobarbital (Pinel et al., 1989; Kim, Pinel, & Roese, 1991), carbamazepine, diazepam, and sodium valproate (Mana, Kim, Pinel, & Jones, 1991) has been shown to be contingent on the administration of convulsive stimulation during periods of drug exposure. For example, Figure 1 illustrates the development of contingent tolerance to the anticonvulsant effects of diazepam (Mana et al., 1991). In this experiment, rats that received diazepam 1 hr before a convulsive stimulation during the tolerance-development phase became significantly tolerant. In contrast, there was no evidence of tolerance in any of the rats that received diazepam 1 hr after a convulsive stimulation. The magnitude and reliability of this effect provide strong support for the drug-effect theory of tolerance.

Another type of support for the drug-effect theory was provided by the innovative study of Mana and Pinel (1987). They showed that the administration of convulsive stimulations in the absence of ethanol is the critical factor in the dissipation of tolerance to the anticonvulsant effects of ethanol on kindled convulsions. In their experiment, there was no loss of tolerance over a 14-day retention interval in rats that received either bidaily ethanol injections each followed by a convulsive stimulation, bidaily ethanol injections alone, or neither ethanol injections nor convulsive stimulations. However, tolerance dissipated completely in rats given bidaily convulsive stimulations alone or bidaily convulsive stimulations each followed by an ethanol injection. Thus, the cessation of ethanol during the dissipation phase was neither a necessary nor a sufficient condition for the dissipation of tolerance to the anticonvulsant effects of ethanol on kindled convulsions. This finding is inconsistent with the drug-exposure theory of tolerance, which predicts that the cessation of drug exposure is the critical factor in the dissipation of tolerance. As predicted by the drug-effect theory, the critical factor in the dissipation of tolerance proved to be the administration of convulsive stimulation in the absence of
FIGURE 1. Contingent tolerance to the anticonvulsant effects of diazepam on amygdalar kindled convulsions in the rat. Rats that received diazepam before each bidaily (one every 48 hr) convulsive stimulation during the tolerance-development phase displayed substantial tolerance to the anticonvulsant effects of diazepam on the tolerance test. In contrast, there was no evidence of tolerance in the control rats that received saline throughout the tolerance-development phase, or in the rats that received diazepam after each convulsive stimulation during the tolerance-development phase, even though the rats in this condition received the same amount of drug exposure as the rats that received diazepam before each bidaily stimulation. [From Mana, 1990. p. 91]
The graph illustrates the development of tolerance to a drug. The y-axis represents the mean forelimb clonus duration in seconds, ranging from 0 to 60. The x-axis indicates the progression from drug free baseline to tolerance test, with stages labeled as 1 to 10.

- **OZP-BEFORE** is represented by solid squares.
- **OZP-AFTER** is represented by open squares.
- **CONTROL** is represented by black circles.

The graph shows an increase in clonus duration over time, reaching a peak and then decreasing during the tolerance test phase.
ethanol. Just as subjects that have adapted to the effects of vision-displacing prisms must experience the effects of the removal of the prisms on visual-motor coordination for their adaptation to dissipate, so too subjects tolerant to the anticonvulsant effects of ethanol must experience seizures in the absence of ethanol for their tolerance to dissipate.

Although Pinel and his colleagues have been the primary force in the study of the contingent development and dissipation of tolerance to anticonvulsant drug effects, recently several prominent groups of investigators have confirmed and extended their findings. Löschner, Rundfeldt, and Honack (1991) demonstrated that the development of tolerance to the anticonvulsant effect of abecarnil in kindled rats was facilitated by the occurrence of seizures during the period of drug exposure. Teitz (1992) reported that kindled rats that received periodic benzodiazepine injections, each followed by an amygdalar stimulation developed tolerance to the anticonvulsant effects of the benzodiazepines, whereas rats that received the benzodiazepine injections after each stimulation did not. Weiss and Post (1991) confirmed that the response contingency is an important factor in both the development and dissipation of tolerance to the anticonvulsant effect of carbamazepine. In their study, only those kindled rats that received a convulsive stimulation after each carbamazepine injection developed tolerance, and this tolerance was subsequently eliminated by a series of drug-free stimulations even when each was followed by a carbamazepine injection, but it was not eliminated by a carbamazepine-free period if no convulsive stimulations were administered during it. These results, together with the findings of Pinel and his colleagues, have provided strong support for the drug-effect theory of tolerance to anticonvulsant drug effects.
(6) Experimental and Clinical Evidence for Tolerance to Anticonvulsant Drug Effects

Evidence that tolerance develops to the anticonvulsant effects of antiepileptic drugs was first reported by Hauptmann (1912)--cited in Frey (1985). Since that time, laboratory experiments have shown that tolerance develops to virtually every antiepileptic drug in clinical use (see Frey, 1987; Kim, 1989). These laboratory demonstrations of tolerance are summarized in Table 1.

Table 2 lists the clinical evidence for the development of tolerance to antiepileptic drugs. It is evident from this table that there are far fewer reports of tolerance in the clinical literature than in the experimental literature. In addition, the nature of the reports is also different. Many of the clinical reports do not explicitly refer to tolerance, instead they comment vaguely on a reduced effectiveness of the antiepileptic medication (Frey, 1987). For those clinical studies that do explicitly report tolerance to antiepileptic medication, a distinction between dispositional and functional tolerance is seldom made (Frey, 1987), and the usual claim is that it develops over months or years. In contrast, tolerance to antiepileptic drugs develops in experimental animals within days or weeks.

The inconsistency between reports of tolerance in the experimental and clinical literature has been attributed to the fact that tolerance is difficult to detect in clinical settings. The following are three of the reasons why tolerance is difficult to detect in clinical settings: 1) physicians commonly increase the dosage of an antiepileptic drug if it does not provide adequate protection against seizures (e.g., see Eadie, 1985; Frey, 1987); 2) physicians commonly practice polypharmacy, that is, they commonly prescribe more than one antiepileptic drug at a time (see Frey, Koella, Meinardi, Schmutz, & Voskuyl, 1986); and 3) the reduced efficacy of antiepileptic drugs in clinical situations can also be attributed to variability in seizure disorders, noncompliance, the growth of the patient, or the presence of metabolites that may interfere with the effect of the parent drug (Frey, 1986). However, despite these difficulties, it is generally accepted that tolerance does
Table 1. Experimental Evidence of Tolerance to Anticonvulsant Effect of Antiepileptic Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type of Tolerance</th>
<th>Reference</th>
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<td>Weiss &amp; Post 1991</td>
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<td>Gent &amp; Haigh 1983</td>
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Table 2. Clinical Evidence of Tolerance to Anticonvulsant Effect of Antiepileptic Drugs

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develop to the effects of most antiepileptic drugs in clinical settings (see Frey, 1987). In fact, the focus of tolerance research has changed recently from the investigation of whether or not tolerance to antiepileptic drugs develops to the investigation of factors that influence its development and dissipation (Frey et al., 1986). This new focus is reflected in the present thesis.

(7) General Rationale and Purpose

The purpose of this thesis was to identify and investigate methods of reducing tolerance to anticonvulsant drug effects. There are two methods of dealing with the problem of tolerance to antiepileptic drugs: 1) by devising methods for reducing the development of tolerance, or 2) by devising methods for facilitating its dissipation once it has developed. Experiments 1 and 2 focused on the first method; Experiment 3 focused on the second.

The impetus and rationale for this thesis came from two sources. The first was the clinical literature on tolerance to antiepileptic drugs. It is now clear that tolerance to antiepileptic drugs is a major problem in the treatment of epilepsy; tolerance to antiepileptic drugs is often so great that initially effective drugs become totally ineffective. Despite the scope and seriousness of this problem, until recently little of the research on tolerance to anticonvulsant drug effects has focused on it. This point was made by Hans Frey (1986), one of the major contributors to the study of tolerance to antiepileptic drugs.

The fact that we still have an only inadequate understanding of the (mainly central) mechanisms that are responsible for what we refer to as functional tolerance (of antiepileptic drugs) makes a basic and thorough concept of this phenomenon still more difficult. And yet, we have to devise ways and means to overcome tolerance lest we lose control of the presently most important target symptom: the seizure. [p. 6]
In conducting the present experiments, my goal was to fill a gap in the research literature on tolerance to antiepileptic drugs and, in doing so, to suggest general strategies for improving the pharmacologic treatment of epilepsy.

The second major impetus for the present experiments came from the drug-effect theory of tolerance. This new way of thinking about drug tolerance, as a biological adaptation to the effects of drugs on ongoing neural activity (rather than to drug exposure per se), seemed like it would be a rich source of hypotheses about the factors that influence the development and dissipation of tolerance. In particular, it seemed that thinking about the factors that influence other forms of biological adaptation might prove to be particularly fruitful, and demonstrating the importance of these factors in the development of tolerance would provide a better understanding of the phenomenon of drug tolerance itself.
GENERAL METHODS

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

Subjects

The subjects in all three experiments were male Long-Evans rats (Charles River, Canada), weighing between 250 and 350 g at the time of surgery. They were individually housed in steel hanging cages in a colony room with an ambient temperature of about 21 degrees C and a 12:12-hr light:dark cycle (lights on at 8:00 a.m.). Purina rat chow and water were available continuously. All experimental manipulations were conducted in the colony room during the light phase of the light-dark cycle.

Surgery

A single bipolar electrode (Plastic Products Company, MS-303-2) was implanted in the left basolateral amygdala of each rat, under sodium pentobarbital anesthesia (65 mg/kg, IP). The electrode tip was aimed at a site 2.8 mm posterior, 5.0 mm to the left, and 8.5 mm ventral to the skull surface at bregma, with the incisor bar set at -3.3 mm (coordinates from Paxinos & Water, 1982). The electrode was secured to the skull with four stainless steel screws and dental acrylic. Powdered tetracycline was sprinkled on the incision before suturing to combat infection.

Kindling

Following a postsurgical recovery period of at least 5 days, each rat was stimulated (1 s, 60 Hz, 400 μA) three times per day, 5 days per week, for 3 weeks. There was a minimum of 2 hr between each stimulation. For each stimulation, each rat was
placed in a plastic box (58 x 58 x 25 cm) containing a thin layer of commercial bedding material, and then the stimulation lead was attached. The stimulation was delivered within a few seconds, and the rat was returned to its cage once all convulsive activity had ceased. As is usual (see Pinel & Rovner, 1978), the initial stimulations produced no behavioral response other than a momentary behavioural arrest, but by the end of this regimen of 45 kindling stimulations, almost every stimulation elicited a clonic convulsion characterized by facial clonus, forelimb clonus, rearing, and loss of equilibrium (a class 5 or 6 convulsion).

Baseline Phase

The baseline phase began approximately 48 hr after the last stimulation of the kindling phase. During the baseline phase, each rat was stimulated five times on a bidaily (one-every-48-hr) schedule. Once initiated, this bidaily schedule was maintained until the end of each experiment. For each convulsion, both the convulsion class and duration of forelimb clonus were recorded; however, as mentioned in the Introduction, the dependent measure in all of the experiments was the duration of forelimb clonus. Despite this focus on forelimb clonus duration, it should be emphasized that the statistical analysis of convulsion class confirmed the conclusions of the present experiments in every way. A vehicle injection was administered 1 hr prior to the fourth baseline stimulation. This was called the saline baseline test. The purpose of this saline baseline test was to assess the effect of the injection procedure on the duration of forelimb clonus elicited by a stimulation. Any rat that displayed less than 20 s of forelimb clonus or displayed running fits on the saline baseline trial was dropped from the experiment. It should be mentioned that from experiment to experiment, the mean forelimb clonus duration on the saline baseline test is always near 40 s. On the fifth baseline trial, all rats received an injection of the drug under investigation 1 hr before a convulsive stimulation. This was called the drug baseline test. The purpose of the drug baseline test was to assess the initial effect of
the drug on the duration of forelimb clonus. Any rat that displayed more than 10 s of
forelimb clonus on this trial was also dropped from the experiment. These rejection
criteria were adopted because the development of tolerance can be clouded by the
inclusion of subjects that do not initially display large anticonvulsant effects. The number
of rats eliminated from each experiment due to these criteria is outlined in the methods
section of each experiment.

Tolerance Development Phase

After the kindling and baseline phases, the rats were assigned to treatment groups
in such a way that the mean durations of forelimb clonus on the saline baseline test and
drug baseline test were approximately equal for all groups. The tolerance-development
phase began 48 hr after the drug baseline test. During the tolerance-development phase,
each rat in the experimental group received an i.p. drug injection 1 hr prior to each bidaily
stimulation, while control rats received a vehicle injection prior to each bidaily
stimulation. The doses of each drug that were used in each experiment were selected on
the basis of previous experiments conducted in this laboratory and on the basis of pilot
observations; we attempted to select the minimum dose that would totally suppress
forelimb clonus on the drug baseline test.

Tolerance Test

Forty-eight hours after the last stimulation of the tolerance-development phase,
each rat received a tolerance test, which was identical to the drug baseline test; each rat
received a drug injection (the same dose as the one used on the drug baseline test) 1 hr
before a convulsive stimulation.
Histology

At the conclusion of each experiment, all rats were sacrificed with CO₂ according to the Canadian Council on Animal Care guidelines. Their brains were removed, preserved in formalin, sliced along the coronal plane, mounted on slides, and then stained with cresyl violet so that the location of the electrode placements could be determined. Figure 2 shows a representative sample of the brains from each experiment. All electrode sites were located in the basolateral amygdala or at its boundary.

Statistical Analyses

The statistical significance of the results in all the experiments of this thesis was evaluated using nonparametric techniques (Siegel & Castellan, 1988) due to extreme differences in variability among the conditions—there was no variability whatsoever in some conditions. The tests used are outlined in the statistics section of each experiment. The basic significance level was set at $p < .05$. Only the data of those rats completing each experiment were subjected to statistical analyses.

The nonparametric statistical tests that were employed to evaluate the results of the present experiments are based on the assumption of random assignment. However, subjects were assigned to groups in the present experiments in a semi-random fashion. Accordingly, the estimates of the statistical significance of differences were all conservative.
FIGURE 2. Representative histological results from the present experiment. The electrode placements in a random sample of 30% of the rats that completed the experiments in this thesis. Most of the electrodes terminated in the basolateral nucleus of the amygdala or at its boundary.
EXPERIMENT 1: THE EFFECT OF DOSE ON THE DEVELOPMENT OF TOLERANCE TO THE ATAXIA-PRODUCING AND ANTICONVULSANT EFFECTS OF PENTOBARBITAL

It is common medical practice to start an epileptic patient off on a low-to-moderate dose of anticonvulsant medication and then to increase the dose incrementally until the convulsions are brought under control (Johannessen, Loyning, & Munthe-Kaas, 1991). However, the effect of this ascending-dose regimen on the development of tolerance to drug effects has not been systematically assessed. The purpose of Experiment 1 was to assess the effects of an ascending-dose regimen on the development of tolerance to the anticonvulsant and ataxia-producing effects of pentobarbital.

My two hypotheses were derived from the drug-effect theory of tolerance. Because the drug-effect theory views drug tolerance as a type of neural adaptation (i.e., as a response to the disruption of normal patterns of neural activity), it suggests that research on other forms of neural adaptation may be a source of insights into drug tolerance. This line of reasoning led to the hypothesis that the progressively increasing disruption of neural activity produced by an ascending-dose regimen might be particularly effective in inducing tolerance, in a manner akin to shaping. It also led me to hypothesize that under some conditions, the effect of dose on the development of tolerance might be different for different drug effects. The drug-effect theory suggests that the effect of dose on tolerance development might be different for the effects of drugs on patterns of neural activity that are elicited only during periods of maximal drug effects (e.g., elicited epileptic activity) than they are for the effects of drugs on patterns of ongoing neural activity (e.g., the neural activity underlying voluntary motor behavior). In the first instance, the drug interacts only briefly with the neural activity underlying the criterion drug effect (less than 1 min for anticonvulsant effects on kindled convulsions); in the second instance, the
drug interacts continuously with the neuronal activity underlying the criterion drug effect as the blood levels gradually rise, hover around asymptote, and then gradually subside.

Both of these hypotheses run counter to the current wisdom about the relation between dose and tolerance development. It is generally believed that higher doses invariably produce greater tolerance (e.g., Aston, 1965; Jorgensen, Fasmer, & Hole, 1986; Kalant & Khanna, 1990; Lê et al., 1984).

Accordingly, in Experiment 1, the effects of dose on the development of tolerance to both the anticonvulsant and ataxia-producing effects of pentobarbital were compared. The anticonvulsant effect of pentobarbital was assessed by measuring the effect of pentobarbital on the duration of forelimb clonus elicited by each stimulation. The ataxia-producing effect of pentobarbital was assessed using the righting test; each rat was placed on its back on a commercial bedding surface, and the latency of its righting reflex (the amount of time it took to roll over onto all four feet) was recorded. This test has been shown to be a reliable and sensitive measure of the ataxia-producing effects of a variety of drugs (e.g., Reeve, Dingwall, Darlington, Scott, Sansom, & Smith, 1992; Wood & Laverty, 1979).

**Methods**

**Subjects**

The subjects were 52 male Long-Evans rats.

**Drugs**

Pentobarbital (BDH Chemicals, purchased in sodium salt form) was dissolved in a vehicle of isotonic saline at a volume of 5 ml/kg. The doses of pentobarbital that were used in this experiment were selected on the basis of the results of Kim (1989).
Baseline Tests

On the saline baseline test, each rat was injected with saline (5 ml/kg) and subsequently given a righting test and a convulsive stimulation. On the drug baseline test 48 hr later, each rat was injected with pentobarbital (20.0 mg/kg) before the righting test and the convulsive stimulation. The righting test was administered 20 min after the injection on both tests, whereas the convulsive stimulation was administered 1 hr after the injection on both tests. The rats that displayed less than 20 s of forelimb clonus on the saline baseline test (n = 8), or more than 10 s of forelimb clonus on the drug baseline test (n = 2) were not studied further. In addition, during the course of the kindling and baseline phases, five rats lost their caps and one developed running fits. Thus, 35 rats remained in the experiment at the start of the tolerance-development phase.

Tolerance-Development Phase

The 35 rats that began the tolerance-development phase of the experiment were divided into four groups. The tolerance-development phase began 48 hr after the drug baseline test. It consisted of 20 bidaily trials in which each rat received either a high dose (50.0 mg/kg; high-dose group; n = 9), a low dose (10.0 mg/kg; low-dose group; n = 9), the saline vehicle (saline group; n = 8), or a series of doses that increased progressively (ascending-dose group, n = 9). Each rat in the ascending-dose group received 10.0 mg/kg on the first trial, and then it received a 1.0 mg/kg dose increase on its next trial every time it demonstrated forelimb clonus. During the tolerance-development phase, all rats received a convulsive stimulation 1 hr after each injection.

Tolerance Test

The tolerance test occurred 48 hr after the last trial of the tolerance-development phase. It was identical to the drug baseline test; each rat received an injection of pentobarbital (20.0 mg/kg) before the righting test and a convulsive stimulation. Again,
the righting test was administered 20 min after the injection and the convulsive stimulation 1 hr after.

Statistics

The Wilcoxon-Signed Ranks Test was used to assess the significance of the within-group differences in forelimb clonus duration between the drug baseline test and the tolerance test. The Kruskal-Wallis one-way analysis of variance was used to assess the significance of the differences among the groups on the tolerance test.

Results

Anticonvulsant Effect

Figure 3 illustrates the mean forelimb clonus durations of all four groups on the test trials: the saline baseline test, the drug baseline test, and the tolerance test. The groups did not differ in their responsiveness to the convulsive stimulation on the saline baseline test, or in their responsiveness to the anticonvulsant effect of pentobarbital on the drug baseline test. However, on the tolerance test, only the rats in the ascending-dose group displayed substantial tolerance to the anticonvulsant effects of pentobarbital. The significance of this result was confirmed by both within-group and between-group analyses. The within-group analyses revealed that the ascending-dose rats displayed significant increases in forelimb clonus duration between the drug baseline test and tolerance test (p < .01), whereas the high-dose rats (p > .65), the low-dose rats (p > .59), and the saline rats (p > .99) did not. The between-groups analysis revealed a significant difference in forelimb clonus duration among the groups (p < .0001) on the tolerance test.
FIGURE 3. The effect of dose on the development of tolerance to the anticonvulsant effect of pentobarbital. There was substantial tolerance development in rats that received an ascending-dose regimen during the tolerance-development phase, whereas there was little evidence of tolerance in rats that received a high-dose regimen, a low-dose regimen, or saline.
Ataxia-Producing Effect

Figure 4 illustrates the percent of rats that righted themselves within 1.5 s on each righting test. The 1.5 s value was chosen as the criterion value because our pilot observations indicated that all undrugged rats normally right themselves within this time. Accordingly, on the saline baseline test, all the rats in each group responded within 1.5 s. In contrast, on the drug baseline test, the proportion of rats in each group that responded within 1.5 s was substantially decreased by pentobarbital. Statistically significant tolerance to this effect developed in only the high-dose group. Overall Chi-Square tests revealed a significant difference in the proportion of rats that responded within 1.5 seconds among the groups on the tolerance test ($p < .02$). Post-hoc Chi-Square tests revealed that on the tolerance test a significantly greater proportion of the high-dose group responded within 1.5 s than of the ascending-dose group ($p < .01$) and the saline control group ($p < .05$). Other differences among the groups were not statistically significant.

Discussion

The results of this experiment confirmed both experimental hypotheses; that an ascending-dose regimen might be particularly effective in inducing tolerance to anticonvulsant drug effects, and that dose can have different effects on the development of tolerance to different drug effects. Rats that received an ascending-dose regimen became significantly more tolerant to the anticonvulsant effects of pentobarbital than did the rats in the other three groups. In contrast, rats that received a high-dose regimen became significantly more tolerant to the ataxia-producing effect of pentobarbital than those in the saline and ascending-dose groups.

Both results were predicted on the basis of the drug-effect theory, which views tolerance as an adaptation to the disruptive effects of drugs on ongoing patterns of neural
FIGURE 4. The effect of dose on the development of tolerance to the ataxia-producing effects of pentobarbital. The rats in the high-dose group displayed significantly more tolerance than did the rats in the ascending-dose group and the saline control group. Differences among the other groups were not significant.
activity. I predicted greater tolerance to the anticonvulsant effect of pentobarbital in the ascending-dose rats because these rats would experience a progressively increasing disruption of the neural activity elicited by each convulsive stimulation as the dose was increased from trial to trial. Shaping is particularly effective in facilitating other forms of neural adaptation, and I assumed that the same would be true of tolerance to anticonvulsant drug effects. Because the effect of the pentobarbital on ongoing motor behaviour was continuously experienced by all subjects following each injection, I predicted that dose would affect this measure in a different way. However, I did not predict in what way its effect would be different. In retrospect, it appears that the greater tolerance to the ataxia-producing effect of pentobarbital in the high-dose rats may be attributed to the progressively increasing disruption of the neural activity underlying the motoric impairment within a single trial; initially the disruptive effect was small, but as the level of drug in the blood rose, the disruption became larger. When shaping is a component of each drug exposure, it appears that consistently high doses are particularly effective in inducing tolerance.

The results for the ataxia-producing effect of pentobarbital seem paradoxical. The rats in the ascending-dose group differed significantly from those in the high-dose group in the proportion of rats responding within 1.5 s on the tolerance test. In contrast, the low-dose rats did not differ significantly from the high-dose rats or the ascending-dose rats in the proportion of rats responding within 1.5 s on the tolerance test. Thus, despite the lack of statistical significance of the differences between the rats in the ascending-dose and low-dose groups, the low-dose rats did appear to display more tolerance to the ataxia-producing effect of pentobarbital, even though the ascending-dose rats received higher doses of pentobarbital. These results do not follow from the hypothesis outlined above that higher doses are particularly effective in inducing tolerance to drug effects that are continuously experienced. However, it may be that in addition to the dose of the drug, the consistency of the dose is also an important factor in the development of tolerance to
these drug effects. For example, both the high-dose and low-dose rats received these
doses consistently on every trial of the tolerance-development phase. Thus, the
progressively increasing disruption of neural activity underlying the motoric impairment
within each trial was the same for every trial. Because the ascending-dose rats did not
receive consistent doses on subsequent trials, the progressively increasing disruption of
neural activity underlying the motoric impairment within each trial was different for
every trial. This suggests that whereas the dose of drug is an important factor (the high-
dose rats displayed more tolerance than the low-dose rats), the manner in which the drug
dose is administered (the low-dose rats displayed more tolerance than the ascending-dose
rats) may also be important in the development of tolerance to drug effects that are
continuously experienced.

Pinel, Kim, Paul, and Mana (1989) previously reported that rats given 10 bidaily
pentobarbital injections (20 mg/kg) 1 hr before a convulsive stimulation did not become
tolerant to the anticonvulsant effect of that dose of pentobarbital. This is consistent with
the present observation that consistently high doses--the 20 mg/kg dose was sufficient to
completely suppress kindled convulsions on every trial in the Pinel et al. study--are less
effective than ascending-dose regimens in inducing tolerance to anticonvulsant drug
effects on kindled convulsions.

As mentioned in the Introduction, a puzzling aspect of drug tolerance is why
tolerance develops to some effects of a drug but not to others, or similarly, why tolerance
develops at different rates for different effects of the same drug. Numerous studies have
reported a differential development of tolerance for different effects of the same drug. For
example, tolerance has been reported to develop independently for the hypothermic and
ataxia-producing effects of ethanol ( Lê et al., 1984), the anesthetic and anticonvulsant
effects of phenobarbital (Dingemanse, Hameter, & Danhof, 1990), the hypothermic and
anticonvulsant effects of pentobarbital (Saunders, Ito, Baker, Hume, & Ho, 1990), the
ataxia-producing and anticonvulsant effects of abecarnil ( Lösch et al., 1991), and the
anticonvulsant and ataxia-producing effects of diazepam, clonazepam, and clobazam (Rosenberg et al., 1989). Similarly, in this experiment, tolerance to the anticonvulsant and ataxia-producing effects of pentobarbital developed independently. A testable hypothesis for these differences can be derived from the drug-effect theory of tolerance and the present results. If tolerance does develop to drug effects, as the drug-effect theory predicts, then the differences in tolerance development for different effects of the same drug may be due to differences in the experiences of different drug effects. For example, some drug effects are normally experienced continuously, whether or not formal tests of the effect are conducted (e.g., hypothermic effects and ataxia), whereas other drug effects are experienced only during the testing procedure (e.g., anticonvulsant effects on elicited seizures). Thus, the crucial factor may lie in the way that different drug effects are experienced, and not in the drug effects themselves. The present results suggest that the development of tolerance to effects that are inevitably experienced at various doses as blood levels increase and decline following each injection is likely to be potentiated by consistent high doses; in contrast, the development of tolerance to effects that are experienced only during a narrow window of time are likely to be facilitated by ascending-dose regimens.

There has been some speculation about potential neural mechanisms underlying differential tolerance development to the effects of the benzodiazepines. Rosenberg, Chiu, and Teitz (1986) have suggested that the development of tolerance to the ataxia-producing and anticonvulsant effects of benzodiazepines are mediated either by two different mechanisms or by the same mechanism acting at two different sites in the nervous system. Conversely, File (1985) has suggested that functional changes in different neurotransmitters downstream from the benzodiazepine-GABA receptor complex may be the mechanisms mediating the different rates of tolerance development to the effects of benzodiazepines. At the present time, there is insufficient evidence to support or refute either of these hypotheses.
EXPERIMENT 2: THE EFFECT OF DOSE ON THE DEVELOPMENT OF TOLERANCE TO THE ANTICONVULSANT EFFECTS OF DIAZEPAM

The purpose of Experiment 2 was to assess the generality of the finding of Experiment 1 that ascending-dose regimens facilitate the development of tolerance to anticonvulsant drug effects. In Experiment 2, the effect of dose on the development of tolerance to the anticonvulsant effects of diazepam was assessed. In contrast to Experiment 1, tolerance to the ataxia-producing effect was not investigated; the doses of diazepam that were used in this experiment did not reliably produce ataxia.

Diazepam was the drug of choice in this experiment because despite its wide therapeutic window, broad spectrum of activity, and lack of aversive side effects, which would seem to make it an ideal drug for the treatment of epilepsy, the clinical use of diazepam as an antiepileptic drug is hampered by the fact that tolerance rapidly develops to its anticonvulsant effects (Haigh & Freeley, 1988). Consequently, the investigation of factors contributing to this tolerance may lead to a more efficacious clinical use of diazepam.

Methods

The methods used in Experiment 2 were similar to those employed in Experiment 1. Accordingly, the methods section for this experiment outlines only the differences between the methods of these two experiments.

Subjects

The subjects were 55 male Long-Evans rats.
Drugs

Diazepam (Hoffman-La Roche, purchased in ampoule form) was mixed in a vehicle of 2% Tween 80 (J.T. Baker Chemical) and isotonic saline at a volume of 5.0 ml/kg. The doses of diazepam that were used in this experiment were selected on the basis of the results of Mana et al. (1991).

Baseline Tests

On the saline baseline test, each rat was injected with saline (5 ml/kg) 1 hr before a convulsive stimulation. On the drug baseline test 48 hr later, each rat received diazepam (3.0 mg/kg) 1 hr before a convulsive stimulation. As in Experiment 1, rats that displayed less than 20 s of forelimb clonus on the saline baseline test (n = 4) or more than 10 s of forelimb clonus on the drug baseline test (n = 7) were not studied further. In addition, 8 rats lost their caps during the kindling and baseline phases of the experiment. Thus, 36 rats began the tolerance-development phase.

Tolerance-Development Phase

The tolerance-development phase consisted of 22 bidaily trials in which each rat received either a high dose of diazepam (10.0 mg/kg, n = 9), a low dose of diazepam (1.0 mg/kg, n = 9), an ascending-dose regimen of diazepam (beginning at 1.0 mg/kg and increasing to 3.0 mg/kg, n = 9), or the saline vehicle (n = 8) 1 hr before a convulsive stimulation. Each rat in the ascending-dose group received a 0.2 mg/kg dose increase on its next trial every time that it demonstrated clonus.

Tolerance Test

The tolerance test was identical to the drug baseline test; each rat received an injection of diazepam (3.0 mg/kg) 1 hr before a convulsive stimulation.
**Stimulation Test**

Approximately 48 hr after the tolerance test, each rat received a convulsive stimulation in the absence of any drug. The purpose of this stimulation test was twofold: first, to ensure that all rats were still responding normally to convulsive stimulation, and second, to determine if there was any evidence of a withdrawal effect. The one rat that did not display a convulsion on the stimulation test was eliminated from the study.

**Statistics**

The statistical analyses were the same as those for Experiment 1. The Wilcoxon-Signed Ranks Test was used to assess the significance of the within-group differences in forelimb clonus duration between the drug baseline test and the tolerance test and between the saline baseline test and the stimulation test and the Kruskal-Wallis one-way analysis of variance by ranks was used to assess the significance of the differences among the groups on the tolerance test and on the stimulation test.

**Results**

Figure 5 illustrates the mean forelimb clonus durations for all four groups on the test trials: the saline baseline test, the drug baseline test, the tolerance test, and the stimulation test. It is readily apparent that the groups did not differ in their responsiveness to the convulsive stimulation on the saline baseline test, or in their responsiveness to the anticonvulsant effect of diazepam on the drug baseline test. However, on the tolerance test, only the rats in the ascending-dose group displayed substantial tolerance to the anticonvulsant effects of diazepam. In addition, on the stimulation test, only the rats in the ascending-dose group and the high-dose group displayed a substantial withdrawal response. The significance of these results was confirmed by both within-group and between-group analyses. A within-group analysis revealed that the ascending-dose rats displayed significant increases in forelimb clonus duration between the drug baseline test
FIGURE 5. The effect of dose on the development of tolerance to the anticonvulsant effect of diazepam. The rats that received an ascending-dose regimen during the tolerance-development phase became significantly tolerant, whereas those that received the high-dose regimen, the low dose-regimen, or saline did not. Paradoxically, despite a lack of tolerance, the high-dose rats displayed a significant withdrawal effect on the stimulation test. The ascending-dose rats also demonstrated a significant withdrawal effect.
and the tolerance test \( (p < .02) \), whereas the high-dose rats \( (p > .32) \), the low-dose rats \( (p > .11) \), and the saline control rats \( (p > .32) \) did not. In addition, a between-group analysis revealed that there was a significant difference in forelimb clonus duration among the groups on the tolerance test. A second within-group analysis revealed that both the ascending-dose group \( (p < .005) \) and the high-dose group \( (p < .008) \), but not the low-dose group \( (p > .05) \) nor the saline control group \( (p > .12) \), displayed significant increases in forelimb clonus between the saline baseline test and the stimulation test. However, a between-group analysis revealed that there were no significant differences in forelimb clonus duration among the groups on the stimulation test \( (p > .09) \).

**Discussion**

The results of this experiment extend the results of Experiment 1. In this experiment, rats that received an ascending-dose regimen during the tolerance-development phase of the experiment became significantly tolerant to the anticonvulsant effects of diazepam, whereas the rats that received a high-dose regimen, a low-dose regimen, or saline did not. As in Experiment 1, these results were predicted by the drug-effect theory of tolerance. They may be attributed to the hypothesis that the rats in the ascending-dose group experienced a progressively increasing disruption of epileptic activity in a manner similar to a shaping procedure. The rats in the other groups did not experience this progressive disruption.

The statistical significance of the results on the stimulation test seem paradoxical; the between-group analysis on the stimulation test indicated that there was no significant group differences in forelimb clonus among the groups on the stimulation test, whereas the within-group analysis indicated that both the high-dose rats and the ascending-dose rats displayed significant increases in forelimb clonus duration from the saline baseline test to the stimulation test. This lack of accordance on these two tests indicates that
whereas there is a suggestion of a withdrawal effect in the high-dose rats and the ascending-dose rats, this result must be interpreted with caution.

This suggested pattern of results following the cessation of the diazepam injections was surprising given the usual assumption about the relation between tolerance and withdrawal effects. The withdrawal effect displayed by the ascending-dose rats was expected because they displayed tolerance on the tolerance test. What was unexpected was the substantial withdrawal effect shown by the high-dose rats, which had not displayed significant tolerance—it is generally believed that tolerance and withdrawal are inextricably related (e.g., Kalant & Khanna, 1990). These results demonstrate that withdrawal effects can occur without any behavioral evidence for the presence of tolerance.

It has been shown that in paradigms involving intermittent drug exposure, the intensity of withdrawal reactions becomes greater with successive cycles of the administration and withdrawal of a drug (Baker & Cannon, 1979; Clemmesen & Hemmingsen, 1984; McCown & Breese, 1990). In this experiment, the rats in both the ascending-dose group and the high-dose group may have experienced a similar successive cycle in that they experienced many brief periods of drug exposure. Consistent with this view is the fact that because the interval between the last drug injection and the stimulation test was 48 hr, the withdrawal effects seen in these rats were probably conditioned (Siegel, 1986). If these rats did experience multiple periods of withdrawal associated with drug exposure, then the association between the withdrawal and the predictive cues of withdrawal would have been strengthened.
EXPERIMENT 3: THE ROLE OF CONVULSIVE STIMULATION ON THE DISSIPATION OF TOLERANCE TO THE ANTICONVULSANT EFFECTS OF DIAZEPAM: TIME COURSE OF THE EFFECT

Experiments 1 and 2 of this thesis investigated one approach to reducing the problem of tolerance to anticonvulsant drug effects: reducing tolerance development by not using ascending-dose protocols. The focus of Experiment 3, on the other hand, was to investigate the second approach to reducing the problem of tolerance to anticonvulsant drug effects: increasing the dissipation of tolerance.

In a previous study of tolerance to the anticonvulsant effects of ethanol, Mana and Pinel (1987) showed that there was no loss of tolerance over a 14-day retention interval in rats that received either bidaily ethanol injections each followed by a convulsive stimulation, bidaily ethanol injections only, or neither ethanol injections nor convulsive stimulations. In contrast, tolerance dissipated completely in rats given bidaily convulsive stimulations or a convulsive stimulation before each bidaily ethanol injection. Thus, the cessation of ethanol alone was neither a necessary nor a sufficient condition for the dissipation of tolerance. The critical factor in the dissipation of tolerance was the experience of convulsions while undrugged.

Mana and Pinel (1987) clearly demonstrated that the experience of convulsive activity in the absence of anticonvulsant drugs is an important factor in the dissipation of tolerance to the anticonvulsant effect of ethanol. However, because the only retention interval that was used in their experiment was 14 days, they provided no information about the rate at which tolerance dissipates: Was one stimulation sufficient to eliminate tolerance or did tolerance decline gradually over the 14-day retention interval? The purpose of Experiment 3 was to extend the findings of Mana and Pinel (1987) to diazepam and to assess the rate at which tolerance declines in rats that receive convulsive stimulations in a drug-free state.
Methods

Subjects

The subjects were 71 male, Long-Evans rats.

Baseline Tests

The saline baseline and drug baseline tests were the same as those in Experiment 2, except that the dose of diazepam was 2.5 mg/kg instead of 3.0 mg/kg.

Tolerance-Development Phase

Beginning 48 hr after the drug baseline test, each rat received 25 bidaily tolerance-development trials in which diazepam (2.5 mg/kg) was injected 1 hr prior to a convulsive stimulation. By the end of the tolerance-development phase, 9 rats had lost their caps, 1 had become ill, 2 did not meet the criterion on the saline baseline test, and 5 did not meet the criterion on the drug baseline test. In addition, 10 rats did not meet the criterion of tolerance--this criterion was a mean duration of forelimb clonus on the last three tolerance-development trials that was at least 50 % of the duration on the saline baseline test. Because the purpose of this experiment was to study the dissipation of tolerance, these 10 rats were eliminated from the experiment. Accordingly, 44 rats entered the tolerance-dissipation phase.

Tolerance-Dissipation Phase

The tolerance-dissipation phase began 48 hr after the last trial of the tolerance-development phase. The remaining 44 rats were divided into nine groups in such a way that the mean forelimb clonus duration elicited over the last three trials of the tolerance-development phase was approximately equal for each group. Of the nine groups, three received no treatment (control group) other than bidaily handling; they were weighed and put briefly into the plastic testing box once (n = 5), three times (n = 5) or seven times (n =
Three other groups received an injection of saline 1 hr before bidaily convulsive stimulations (stim-only group) for one trial (n = 5), three trials (n = 4), or seven trials (n = 5). The final three groups received an injection of diazepam 1 hr before bidaily convulsive stimulations (stim-before-dz group) for one trial (n = 5), three trials (n = 5), or seven trials (n = 5). Accordingly, the design was a 3x3 factorial with 3 retention intervals and 3 stimulation and drug conditions.

**Tolerance-Retention Test**

Each rat received a tolerance test 48 hr after the last trial of its tolerance-dissipation phase. Thus, tolerance tests were administered 4 days, 8 days, or 16 days after the final tolerance-development trial. Each tolerance test was identical to the drug baseline test; each rat received an injection of diazepam (2.5 mg/kg) 1 hr prior to a convulsive stimulation. The purpose of each tolerance test was to assess the degree to which tolerance to the anticonvulsant effects of diazepam had dissipated.

**Statistics**

As in Experiments 1 and 2, the significance of the results was assessed using non-parametric techniques. The Wilcoxon Signed-Ranks Test was used to assess the significance of the within group differences in the retention of tolerance; the mean duration of forelimb clonus elicited by each rat on the last three trials of the tolerance-development phase was compared to the duration of forelimb clonus elicited from that rat on its tolerance-retention test. The Kruskal-Wallis one-way analysis of variance was used to assess the significance of the differences in forelimb clonus duration among the groups after each retention interval.
Results

Figure 6 illustrates the mean forelimb clonus durations for all the rats on the saline baseline test, the drug baseline test, and the acquisition of tolerance during the 25-trial tolerance-development phase. It also shows the dissipation of tolerance for individual groups after the 4-day retention interval, the 8-day retention interval, and the 16-day retention interval. It is obvious not only that tolerance dissipated in the rats that received a convulsive stimulation only and the rats that received a convulsive stimulation before an injection of diazepam, but also that this dissipation was gradual. In contrast, there was no decline of tolerance in the rats that received no stimulations or diazepam during the retention intervals. Instead these rats showed an increase in the amount of forelimb clonus displayed after the retention interval.

These results were statistically significant. After the 4-day retention interval, none of the groups displayed any significant dissipation of tolerance. After the 8-day retention interval, the rats in the stim-before-dz group displayed significantly less forelimb clonus than they had at the end of the tolerance-development phase (p < .05), whereas those in the stim-only group (p > .07) did not. Paradoxically, the rats in the control group displayed significantly more forelimb clonus after the 8-day retention interval (p < .04). After the 16-day retention interval, the rats in both the stim-before-dz group (p < .05) and stim-only group (p < .05) demonstrated significantly less forelimb clonus, whereas those in the control group did not (p > .22). Similar results were obtained with between-group statistical analyses. There were no significant differences in forelimb clonus among the groups after the 4-day retention interval (p > .16). In contrast, there were significant group differences after both the 8-day (p < .02) and the 16-day intervals (p < .006).
FIGURE 6. The effect of convulsive stimulations on the dissipation of tolerance to the anticonvulsant effect of diazepam. Tolerance did not dissipate in rats that received no diazepam over either a 4-day, 8-day, or 16-day retention interval if they were not stimulated during this interval (control). In contrast, tolerance dissipated monotonically in rats that received bidaily stimulations during the retention interval, even if they received diazepam after each stimulation.
Discussion

The results of Experiment 3 confirm the previous finding of Mana and Pinel (1987) that the administration of convulsive stimulations in the absence of drug is critical for the dissipation of tolerance to anticonvulsant drug effects. They also provide the first experimental evidence that this dissipation occurs gradually. Tolerance to the anticonvulsant effects of diazepam did not decline in rats that received neither convulsive stimulations nor diazepam during the 16-day retention interval. In contrast, tolerance declined steadily over the 16-day retention interval in rats exposed to convulsive stimulations alone, or to convulsive stimulations before diazepam injections during the interval. Thus, the withdrawal of diazepam alone had no effect whatsoever on the dissipation of tolerance. The response contingency was the critical factor in the dissipation of tolerance; rats exposed to the exact same regimen of bidaily diazepam injections that made them tolerant, lost their tolerance if the stimulations were administered before, rather than after, the injection.

The finding that tolerance to the anticonvulsant effects of diazepam declines gradually suggests that the adaptation that occurs during the development of tolerance cannot be overcome by the experience of 1 or 2 convulsions in the absence of drug. However, it may not suggest that this adaptation occurs in a slow, steady progression. Despite the fact that the mean duration of forelimb clonus indicated a such a slow, steady decline of tolerance, the data of individual rats do not tell such a clear story. For example, of the five rats that received a convulsive stimulation before diazepam during the 8-day retention interval, only two actually displayed a substantial reduction in forelimb clonus duration, whereas one displayed a small reduction and two others displayed no reduction at all. This suggests that individual rats may in fact experience a sudden loss of tolerance. If this sudden loss occurred at different times for different rats, then the group mean would reflect a gradual loss of tolerance. Thus, the use of the term gradual to describe the decline of tolerance means that 1 or 2 convulsive stimulations in the absence of drug did
not cause any dissipation of tolerance; however, once enough convulsive stimulations were administered to cause a decline in tolerance, it did not necessarily dissipate at a slow, steady rate.

At the 8-day retention interval, the rats in the control group (no treatment other than handling) displayed a significant increase in forelimb clonus. It is possible that this increase was due to a withdrawal effect, however, because the rats in tested after the 4-day retention interval did not display an increase of the same magnitude, this explanation seems unlikely. If the increased forelimb clonus was due to a withdrawal effect, then it should also have been greatest at the shortest retention interval. Alternatively, the increase in forelimb clonus displayed after the 8-day retention interval could be explained by the data of individual rats. Two of the five rats in this group displayed large increases in forelimb clonus duration whereas the other three rats did not. Because the number of rats in each group was small in this experiment, the unusually high scores from these two rats are directly reflected in the increased group mean. If this experiment was repeated with the standard 10 to 12 rats in each group, then unusually high forelimb clonus durations would not affect the group mean to such a large extent.
GENERAL DISCUSSION

The general purpose of this thesis was to identify and investigate factors that can reduce tolerance to anticonvulsant drug effects. Experiments 1 and 2 showed that ascending-dose regimens facilitate the development of tolerance to anticonvulsant drug effects; Experiment 1 also showed that dose can have different effects on the development of tolerance to different drug effects. Experiment 3 showed that the administration of convulsive stimulations in the absence of drug results in a gradual dissipation of tolerance, even in subjects that continue to receive drug injections. Because the drug-effect theory predicted these results, they provide general support for it.

The General Discussion of this thesis is divided into 5 sections. Section 1 deals with Experiments 1 and 2, and Section 2 deals with Experiment 3. Sections 3 and 4 present the theoretical and clinical implications of the present research, respectively. And finally, Section 5 discusses the conclusions and future directions of this work.

(1) General Discussion of Experiments 1 and 2

The purpose of Experiments 1 and 2 was to investigate the effect of drug dose on the development of tolerance to anticonvulsant drug effects. In both experiments, amygdala-kindled rats received either the same high dose, the same low dose, an ascending-dose regimen, or injections of saline during the tolerance-development phase of the experiment. In Experiment 1, significant tolerance developed to the anticonvulsant effect of pentobarbital only in the ascending-dose rats, whereas significant tolerance to the ataxia-producing effect of pentobarbital developed only in the high-dose rats. In Experiment 2, only the rats that received an ascending-dose regimen displayed tolerance to the anticonvulsant effects of diazepam. Together, the results of these experiments confirmed both experimental hypotheses: that an ascending-dose regimen might be
particularly effective in inducing tolerance, and that dose can have different effects on the development of tolerance to different drug effects.

These results are inconsistent with widely held assumption that high doses are inevitably associated with greater tolerance. If this assumption were true, then the rats in the high-dose groups of Experiments 1 and 2 should have developed more tolerance to anticonvulsant drug effects because they received more drug than did the rats in the other groups. Clearly, they did not. Figures 3 and 5 show that in both experiments, the ascending-dose rats developed substantially more tolerance to anticonvulsant drug effects than the high-dose rats and the low-dose rats. Moreover, the low-dose rats developed slightly more tolerance than the high-dose rats. However, these results were predicted by the drug-effect theory of tolerance. The greater tolerance to the anticonvulsant effects of both pentobarbital and diazepam in the ascending-dose rats may be attributed to the fact that these rats repeatedly experienced the disruptive effect of the drug on the neural activity which occurs with convulsions. These disruptions became progressively larger as the dose of drug was increased. Thus, the development of tolerance in these rats was akin to a shaping procedure.

The only other report of the effects of an ascending-dose regimen confirms the results of Experiments 1 and 2. Killam, Matsuzaki, and Killam (1973) studied the anticonvulsant effects of two benzodiazepines (diazepam and RO 5-4023) on photically elicited seizures in a strain of seizure-prone baboons, *Papio papio*. They found that low doses of either drug, which initially blocked epileptic activity, soon became ineffective. In an attempt to find an acceptable therapeutic dose, they increased the dose. They unexpectedly found that increasing the dose provided only temporary relief, and the dose had to be increased again. In contrast, moderately high doses administered from the start remained effective for the duration of the study. Because I did not discover the Killam et al. finding until after I had completed Experiments 1 and 2, in a sense it provides
independent confirmation of my hypothesis that ascending-dose regimens facilitate the development of tolerance to anticonvulsant drug effects.

There is evidence to support the view that higher doses produce greater tolerance (see Kalant & Khanna, 1990). For example, Aston (1965) found that the magnitude of tolerance to the anesthetic effect of pentobarbital is a positive monotonic function of dose; Lê, Khanna, and Kalant (1984) found that tolerance to both the hypothermia and the motor impairment produced by ethanol was greater at higher doses; and Jorgensen, Fasmer, and Hole (1985) found that higher doses produced more tolerance to the inhibitory effect of ethanol on the tail-flick reflex. The difference between these results and those of the present experiments may be explained by a difference in the expression of the particular drug effect under investigation. For example, the hypothermic and ataxic effects of ethanol are experienced by rats continuously after administration of the drug. Conversely, the rats in Experiments 1 and 2 of this thesis experienced the anticonvulsant effects of pentobarbital and diazepam only when a convulsive stimulation was given during periods of drug exposure. Support for this interpretation comes from the results of Experiment 1 in which only rats exposed to an ascending-dose regimen became significantly tolerant to pentobarbital's anticonvulsant effect, whereas only rats exposed to a high-dose regimen became significantly tolerant to pentobarbital's ataxia-producing effect.

A problem with studies that examine the relation between dose and tolerance is that the detection of tolerance in the subjects that receive the high 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, in this experiment, the rats in the high-dose group displayed significant tolerance to pentobarbital's ataxia-producing effect, but not to its anticonvulsant effect. If drug accumulation accounted for the lack of tolerance to
pentobarbital's anticonvulsant effect in the high-dose rats, then it should also have obscured the development of tolerance to pentobarbital's ataxia-producing effect.

The therapeutic implications of Experiments 1 and 2 must be viewed with caution. The present results suggest that consistently high doses do not lead to substantial tolerance to the anticonvulsant effects of pentobarbital and diazepam. However, there is no way of knowing whether or not more tolerance would have developed if the treatment period had been extended. In addition, the lack of tolerance in the high-dose rats may have been due to a baseline problem; tolerance in the high-dose rats may have been revealed if the test dose had been smaller.

(2) General Discussion of Experiment 3

The purpose of Experiment 3 was to twofold: to determine whether the elicitation of convulsions in the absence of drug is the critical factor in the dissipation of tolerance to diazepam and to determine the time course of the dissipation of tolerance to the anticonvulsant effects of diazepam. Experiment 3 was a direct extension of Mana and Pinel's (1987) study of the dissipation of tolerance to the anticonvulsant effects of alcohol.

The results of Experiment 3 confirmed and extended Mana and Pinel's finding; the critical factor in the dissipation of tolerance to the anticonvulsant effects of diazepam proved to be convulsive stimulation in the absence of the drug. In addition, I found that the dissipation occurred at a gradual rate (after more than 1 or 2 convulsive stimulations) over the 16-day retention interval. During this retention interval, the cessation of diazepam administration was neither necessary nor sufficient for the dissipation of tolerance to diazepam's anticonvulsant effect, and the continuation of diazepam administration was neither necessary nor sufficient for the retention of this tolerance once it had developed. Instead, the critical factor in the dissipation of tolerance was the elicitation of the criterion response--convulsive activity--in the absence of diazepam. If
convulsions were elicited in the absence of diazepam, then tolerance to diazepam's anticonvulsant effect dissipated over the 14-day retention interval; if this condition was not met, then there was no dissipation of tolerance.

These results cannot be accounted for by the implicit drug-exposure theory of tolerance. If drug exposure is the critical factor in the development of tolerance to anticonvulsant drug effects, then the cessation of drug exposure should be sufficient for the dissipation of tolerance to anticonvulsant drug effects. This was clearly not the case in Experiment 3--there was no dissipation of tolerance whatsoever in the control groups. Instead, the results this experiment are consistent with the drug-effect theory of tolerance. Just as the drug-effect theory predicts that the criterion response (the convulsion) must be experienced under the influence of the drug for tolerance to develop, it also predicts that the dissipation of tolerance is contingent on the experience of the criterion response (the convulsion) in the absence of drug.

Similar support for the importance of the response contingency in the dissipation of tolerance has been provided by other studies--in addition to the experiment of Mana and Pinel. For example, it has been shown that rats will not lose their tolerance as rapidly to the effects of tetrahydrocannabinol on operant responding unless they can perform the response in the absence of drug (Manning, 1974), to amphetamine's anorectic effect unless they eat in the absence of the drug (Poulos, Wilkinson, & Cappell, 1984), to scopolamine's adipsic effect unless they are allowed to drink in the absence of the drug (Poulos & Hinson, 1984), or to carbamazepine's anticonvulsant effect unless they experience convulsions in the absence of drug (Weiss & Post, 1991).

(3) Theoretical Implications

Although the experiments in this thesis were derived from the drug-effect theory of tolerance, they were not designed to provide critical tests of this theory. Nevertheless,
their results have provided general support for the drug-effect theory by showing that it can direct the study of drug tolerance into fruitful new areas.

According to the drug-effect theory, a drug-produced disruption in normal patterns of neural activity causes the neural system to adapt such that the disruption of neural activity is reduced. Once this adaptation has occurred, a disruption of the changed pattern of neural activity occurs if the drug is removed, and the neural system readapts to this disruption, such that the original effect of the drug on the neural activity is restored. In the Introduction, the point was made that to expect tolerance to develop to a drug's effects on a response that is not performed during periods of drug exposure is like expecting adaptation to the disruptive effects of laterally-displacing prisms on visuomotor performance to occur in the absence of the opportunity to perceive the disruption. Neither the adaptation to the disruptive effects of visual displacement on visuomotor coordination nor the adaptation to the disruptive effects of anticonvulsant drugs on kindled seizures can occur unless the disruptive effects are actually experienced.

The relevance of this displaced-vision analogy to the results of the present experiments is as follows: A subject that wears laterally-displacing prisms that displaces vision slightly will adapt quickly to this displacement. If the degree of displacement is then increased a little further, adaptation will again occur quickly. After several repetitions of this cycle, the subject will have adapted to a large displacement via several small adaptations, similar to a shaping procedure. Similarly, Experiments 1 and 2 demonstrate that tolerance develops more quickly in rats exposed to a progressively increasing series of drug doses. In contrast, once a subject has adapted to the effects of displacing prisms, removing them produces another change in the relation between visual and motor feedback to which the subject must adapt. Just as the visual perception of the disruptive effect of the prisms on visuomotor coordination is necessary for the adaptation to the introduction of the prisms, the visual perception of visuomotor coordination with them off is necessary for adaptation to their removal. Similarly, Experiment 3
demonstrates that the critical event in the dissipation of tolerance to the anticonvulsant effects of diazepam is the experience of convulsions in the absence of diazepam. The dissipation is not a simple consequence of withdrawal of the drug; the dissipation of tolerance requires that the absence of the drug effect on the criterion response (the convulsive response) can be experienced.

This idea that the development and dissipation of tolerance to anticonvulsant drug effects is dependent on the activity of the subject bears a striking resemblance to an idea that has recently gained prominence in many areas of neuroscience research: that neural plasticity is a function of activity-dependent change. For example, the importance of activity-dependent change has been illustrated for the innervation of the nicotinic acetylcholine receptors on muscle fibres (e.g., Landmesser, 1980; Lømø & Rosenthal, 1972), the development of ocular dominance columns in visual cortex (e.g., Bear, Kleinschmidt, Gu, & Singer, 1990; Weisel & Hubel, 1965), and the development of long-term potentiation (e.g., Bliss & Lømø, 1973; Bliss & Lynch, 1988; Gustafsson & Wigstrom, 1988). In recognition of this resemblance, Mana (1990) suggested a model of activity-dependent change in the development of tolerance to anticonvulsant drug effects. In his model, tolerance is a function of both the pharmacologic factors associated with drug exposure (e.g., dose; schedule of administration) and the activity of the nervous system during periods of drug exposure. Thus, the relation between pharmacologic factors (P) and neural activity (N) in the development of tolerance (T) can be represented by the expression: \( T = P \times N \). In this model, a basal amount of neural activity is assumed to be necessary for the drug effects to express themselves and functional tolerance to develop, regardless of the pharmacological conditions. If sufficiently high doses are administered to eliminate activity in the appropriate neural circuits, then tolerance would not develop. Similarly, in order for tolerance to decline, the expression of neural activity in the absence of any pharmacologic factors (i.e., drug) is necessary.
(4) Clinical Implications

The main purpose of this thesis was to identify and investigate factors that could reduce tolerance to anticonvulsant drug effects. However, it is important to realize that there are several methodological differences between the experiments in this thesis and the clinical situation. In the experiments in this thesis, the subjects were rats, not humans; the convulsions were elicited, not spontaneously; and the drugs were administered at spaced time intervals, not at brief intervals designed to maintain a certain blood plasma level of the drug. Thus, the potential clinical implications of this work should be interpreted with caution.

There are two main clinical implications arising from the experiments in this thesis. The first clinical implication stems from the observation in Experiments 1 and 2 that the development of tolerance to anticonvulsant drug effects may be facilitated in subjects that experience a progressively increasing disruption of the neural activity elicited by each convulsive stimulation. It is common clinical practice in the treatment of epilepsy to start a patient off on a low dose of anticonvulsant medication, and then to increase the dose incrementally once seizures begin to recur. However, this procedure may facilitate the development of tolerance to the anticonvulsant effects of antiepileptic drugs because the patient would be experiencing a progressive disruptive effect of the drug on seizure activity. An alternative strategy to the usual ascending-dose protocol would be to give the patient a relatively high initial dose to ensure that the convulsions are less likely to be experienced; the drug-effect theory suggests that this might slow or prevent the development of tolerance to antiepileptic drugs. This strategy would result in the occurrence of aversive side effects, but it does have several advantages over the current procedure for administering antiepileptic drugs. One advantage is that because patients are started on a large dose, the final effective therapeutic dose that is achieved may be lower since less tolerance has developed (Killam et al., 1973). A second advantage of administering initial high doses of anticonvulsant medication is that this
procedure would bring the seizures under control more quickly. There is evidence that the repeated occurrence of seizures may result in brain damage (Dam, 1982); this has been demonstrated in human epileptics and in laboratory animals. In addition, seizures may result in some alteration in the brain which makes the future occurrence of seizures more likely. There is evidence of this from a variety of experimental convulsant models in animals (Pinel, Van Oot, & Mucha, 1975). It has also been suggested that this type of "kindling" effect is a mechanism in the development of the epileptic syndrome (Resor & Nutt, 1992). Thus, quick control of seizures may be of paramount importance for epileptic patients.

The second clinical implication stems from the observation in Experiment 3 that the occurrence of convulsive activity in the absence of drug was the critical factor in the dissipation of tolerance to anticonvulsant drug effects. This observation may be applicable to epileptic patients who are drug refractory, suggesting the possible clinical utility of a period of drug discontinuation. The occurrence of seizures during this drug-free interval might reinstate drug responsivity. Despite the obvious ethical problems associated with this procedure, there is some clinical support for it. Gastaut and Low (1979) have reported that the anticonvulsant effect of clobazam was reinstated after a period of interrupted treatment in which seizures were allowed to recur. Pazzaglia and Post (1992) have provided similar evidence with a different drug effect, the antinociceptive effects of carbamazepine in the treatment of trigeminal neuralgia. They reported a single-case study in which the patient became contingently tolerant to the antinociceptive effects of carbamazepine. After a period of carbamazepine discontinuation, treatment was initiated again, and the patient displayed no evidence of the tolerance that had been previously demonstrated. These results suggest that the practice of discontinuing anticonvulsant drug therapy for short periods of time may in fact, lead to fewer problems with tolerance development. However, there is a problem with this treatment strategy. The implementation of a period of drug discontinuation
could reduce the development of tolerance, but it would also result in the patient experiencing seizures. As mentioned above, the occurrence of seizures may worsen the epileptic syndrome by causing brain damage and by promoting the future occurrence of seizures.

(5) Conclusions and Future Directions

The treatment of epilepsy is hampered by the fact that tolerance develops to the anticonvulsant effects of almost every antiepileptic drug currently in use. In this thesis, the drug-effect theory provided the framework by which factors influencing tolerance to anticonvulsant drug effects could be investigated. Experiments 1 and 2 provided evidence that the dose of drug plays an important role in the development of tolerance to anticonvulsant drug effects—as ascending-dose sequences greatly facilitated the development of tolerance. Experiment 3 provided evidence that the occurrence of convulsions in the absence of drug resulted in the gradual decline of tolerance to anticonvulsant drug effects.

The results of these experiments are important for two main reasons. First, they suggest practical strategies for reducing the problem of tolerance to anticonvulsant drug effects in the treatment of epilepsy. As mentioned in the Introduction, this type of research is sorely lacking in the literature. And second, they provide general support for the drug-effect theory of tolerance by showing that the drug-effect theory can lead one into fruitful new areas of research.

For example, the present findings have raised two interesting questions that could be the focus of future research. The first one involves the relation between tolerance and withdrawal. It is generally agreed that these two phenomena reflect a common adaptation, so that the physiological change that is presumed to produce tolerance is also assumed to be responsible for withdrawal effects (Balster, 1984). However, in Experiment 2, rats that received a high dose of diazepam did not develop a significant amount of tolerance to the
anticonvulsant effects of diazepam, yet they displayed a substantial withdrawal effect once the diazepam was discontinued. Furthermore, in Experiment 3, there was no evidence of a withdrawal effect in tolerant rats that received convulsive stimulations alone during the retention interval. These observations suggest that the hypothesis that tolerance and withdrawal are inextricably related may have to be re-evaluated.

The second interesting question raised by the present findings involves the idea presented in Experiment 1 that the development of tolerance to drug effects on punctate patterns of behavior, such as convulsions, may be different than the development of tolerance to drug effects on continuous patterns of behavior, such as motor activity. In the first case, the drug interacts with the neural activity causing a convulsion for only a few seconds, whereas in the second instance, the drug interacts continuously with the neural activity responsible for motor impairment. This distinction is one possible explanation for the dissociation of tolerance development between the anticonvulsant and ataxia-producing effects of pentobarbital seen in Experiment 1. This variable has never been studied—or to my knowledge, mentioned—in the tolerance literature.

In summary, the importance of the drug-effect theory is underlined by the fact that the above-mentioned questions would not have been asked if drug tolerance was only thought of in terms of the implicit drug-exposure theory of tolerance. In that sense, the ability of the drug-effect theory to spawn these questions is a significant step toward establishing its value.
REFERENCES


