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Date September 7, 1990

DE-6 (2/88)

ABSTRACT

The development of tolerance to anticonvulsant drug effects has traditionally been studied in terms of pharmacological variables associated with the drug itself; for example, the dose or the schedule of administration. This type of tolerance is referred to as pharmacologic drug tolerance. In contrast, we have demonstrated that the development of tolerance to ethanol's anticonvulsant effect is contingent upon the adminstration of convulsive stimulation during periods of ethanol exposure; we refer to this as contingent drug tolerance.

The purpose of the first two experiments in the present thesis was to extend the phenomenon of contingent tolerance to the anticonvulsant effects of three clinically relevant antiepileptic drugs: carbamazepine (CBZ), diazepam (DZP), and sodium valproate (VPA). In Experiment 1, kindled rats that received an injection of CBZ (70 mg/kg, IP), DZP (2 mg/kg, IP), or VPA (250 mg/kg, IP) 1 hr before each of 10 bidaily (one every 48 hr) convulsive stimulations displayed a significant amount of tolerance to the drugs' anticonvulsant effects on the tolerance test trial; in contrast, there was no evidence of tolerance in the rats from the three vehicle control groups. In Experiment 2, the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA, administered on a bidaily basis, was shown to be contingent upon the administration of convulsive stimulation during the periods of drug exposure. Kindled rats in the three drug-before-stimulation groups rapidly developed tolerance to the

anticonvulsant effects of CBZ, DZP, and VPA; in contrast, there was no evidence of tolerance in the respective drug-after-stimulation groups, despite the fact that they had the same drug history.

The purpose of the final three experiments was to compare contingent and pharmacologic tolerance to the anticonvulsant effects of DZP. Experiment 3 replicated earlier demonstrations of pharmacologic tolerance to DZP's anticonvulsant effect; kindled rats that received chronic DZP (2 mg/kg, every 8 hr, for 10 days) developed tolerance to the drug's anticonvulsant effect even though they did not receive convulsive stimulation during the periods of drug exposure. In Experiment 4, the rate of dissipation of pharmacologic and contingent tolerance to DZP's anticonvulsant effect was compared. Pharmacologic tolerance gradually dissipated over the 16-day retention interval; in contrast, there was no evidence of dissipation of contingent tolerance after 16 days of drug withdrawal. These data suggest that different physiological changes are responsible for pharmacologic and contingent tolerance to DZP's anticonvulsant effect. This conclusion was supported by the results of Experiment 5, in which a single injection of the benzodiazepine receptor antagonist RO 15-1788 24 hr prior to a tolerance-retention test trial significantly reduced the expression of pharmacologic tolerance, but not contingent tolerance, to DZP's anticonvulsant effect.

The results of these five experiments make two general

points. First, concurrent convulsive stimulation can have an important effect on the development of tolerance to the anticonvulsant effects of antiepileptic drugs. And second, there are significant differences in the physiological changes responsible for the development and the dissipation of contingent and pharmacologic tolerance to DZP's anticonvulsant effect. Because traditional theories do not address these differences, a new model of contingent and pharmacologic tolerance is presented.

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ACKNOWLEDGEMENTS

I thank Dr. John P.J. Pinel for his support, his confidence, and his friendship over the last 6 years, and for his contributions in word and thought to much of what I have done during that time. I thank Dr. Donald M. Wilkie for his doorway and our conversations, and for setting the pace. I also thank Dr. Richard C. Tees, Dr. Catherine Rankin and Dr. William J. Jacobs for their support and insights; C. Kwon Kim for his inspiring assistance in all phases of the first 2 experiments and for hours of discussion about things contingent; Brian Moorehead and Keith Waldron for keeping things functional; Lucille Hoover for her assistance during the course of the experiments; and C.H. Jones for his contribution to Experiment 2. I thank Dr. Robert Douglas for thoughts about the possible bases of activity-dependent change and for his contributions to this thesis; Dr. Darren Lehman, Dr. Catherine Rankin, Dr. Ken Baimbridge, Dr. Ralph Hakstian, and Dr. Andrew J. Goudie for their contributions to this thesis; and the Medical Research Council of Canada for its support. And I thank Fin.

I. INTRODUCTION

In the last six years, Pinel and his associates have published a series of papers demonstrating that the development of tolerance to ethanol's anticonvulsant effect on kindled rats is greatly influenced by the administration of convulsive stimulation during the periods of ethanol exposure (e.g., Pinel, Colbourne, Sigalet & Renfrey, 1983; Pinel, Mana, & Renfrey, 1985; Pinel, Kim, Paul, & Mana, 1989). In each of these papers, tolerance to the anticonvulsant effect of ethanol developed only when kindled rats received convulsive stimulation during each period of ethanol exposure; kindled rats that were unstimulated, or stimulated prior to each period of ethanol exposure, demonstrated little tolerance on the test trial even though these rats received exactly the same ethanol exposure. We have referred to the tolerance that develops to ethanol's anticonvulsant effect only when convulsive stimulation is administered during periods of ethanol exposure as <u>contingent tolerance</u> (Pinel et al., 1985; Pinel & Mana, 1986; see also Carlton & Wolgin, 1971) because the development of tolerance is contingent upon convulsive stimulation during periods of ethanol exposure rather than upon ethanol exposure per se.

Although the development of tolerance to ethanol's anticonvulsant effects is clearly influenced by the administration of convulsive stimulation during the periods of ethanol exposure, to date there has been no systematic attempt to determine whether convulsive stimulation has a similar effect on

the development of tolerance to the anticonvulsant effects of clinically relevant antiepileptic drugs. This is particularly noteworthy in light of the fact that there is experimental evidence of tolerance to the anticonvulsant effects of almost every antiepileptic drug currently in clinical use (see Frey, 1987). We have referred to this type of tolerance, which develops in the absence of concurrent convulsive stimulation, as pharmacologic drug tolerance (Mana, Kim, Pinel, & Jones, submitted; see also Jørgensen, Fasmer, & Hole, 1986).

The present experiments were conducted for two primary purposes: (1) to assess the role of convulsive stimulation in the development of tolerance to the anticonvulsant effects of antiepileptic drugs, and (2) to compare contingent and pharmacologic tolerance to anticonvulsant drug effects. The first two experiments were designed to assess the degree to which the development of tolerance to the anticonvulsant effects of three clinically relevant antiepileptic drugs--carbamazepine, diazepam, and sodium valproate--is contingent upon the concurrent administration of convulsive stimulation with drug exposure. The three remaining experiments compared contingent and pharmacologic tolerance to the anticonvulsant effects of diazepam. Accordingly, the first three sections of the Introduction focus on the following 3 topics: (1) the phenomenon of drug tolerance, with an emphasis upon the traditional concept of pharmacologic drug tolerance; (2) the contingent tolerance phenomenon, with an emphasis upon our research on contingent tolerance to ethanol's

anticonvulsant effects; and (3) tolerance to the anticonvulsant effects of antiepileptic drugs, with an emphasis upon the three drugs--carbamazepine, diazepam, and sodium valproate--that were the focus for the present experiments. The fourth and final section of the Introduction presents the general purpose for the five experiments that compose this thesis.

1. Drug Tolerance

Because it is an interesting example of biological adaptation (see Cappell & LeBlanc, 1979) and because of its hypothetical relation to the phenomena of drug dependence, withdrawal, and abuse (Haefly, 1986; Kalant, 1985; Siegel & MacRae, 1984), tolerance is one of the most widely studied drug-related phenomena (see Goudie & Emmett-Oglesby, 1989a). Yet our understanding of tolerance remains at an elementary level.

Definition

Drug tolerance is usually defined as a decrease in the effect of a given dose of a drug that occurs as the result of previous exposure to the drug. In many instances, the development of tolerance to a drug effect results in a shift in the dose-response curve for that effect to the right (so that the maximum drug effect can still be achieved if the drug dose is increased), but in other cases the development of tolerance flattens the dose-response curve (so that there is a decrease in the maximal drug effect, regardless of dose; e.g., Haigh, Gent,

Garrat, Pullar, & Feely, 1988; Lê, Khanna, Kalant, & Grossi, 1986). Kalant (1989) has proposed that the type of change seen in the dose-response curve following the development of tolerance to a drug's effects provides an insight into the drug's site of action. According to Kalant (1989), a parallel rightward shift in the dose-response curve following the development of tolerance is typical of drugs with a nonspecific site of action in the nervous system (e.g., ethanol, general anaesthetics), whereas a rightward shift and flattening of the dose-response curve is typical of drugs that produce desensitization or down-regulation of a stereospecific, receptor-mediated mechanism of action (e.g., opiates, benzodiazepines).

Although tolerance develops to the effects of many drugs, it does not develop to the effects of all drugs. And it does not necessarily develop to all of the effects of a particular drug; exposure to a particular drug may lead to the development of tolerance to some of its effects (often with a different time course for each effect; e.g., Löscher & Hönack, 1989; Rosenberg, Chiu, & Tietz, 1986), while others may be unchanged or even increased in magnitude (e.g., Woolverton, Kandel, & Schuster, 1978; see Lê & Khanna, 1989). This picture is further complicated by the fact that the development of tolerance to one of a drug's effects may be obscured by the development of tolerance or sensitization to another effect of the same drug. For example, Mucha, Kalant, and Kim (1987) found that tolerance develops to morphine's hypothermic effect (which is apparent soon

after administration of the drug) much more rapidly than to its hyperthermic effect (which is not apparent until some time after the drug is administered). As a result, subjects receiving repeated morphine injections appear to develop tolerance to the drug's hypothermic effects and sensitization to its hyperthermic effects (because the fast-developing tolerance that develops to morphine's hypothermic effect allows the hyperthermic effect to express itself earlier and to a greater degree), when in fact tolerance is developing to both of these thermic effects of morphine, but at a different rate.

Biological Mechanisms of Tolerance Development

There are two general types of biological change to which tolerance to a particular drug effect can be attributed: dispositional change or functional change. Although a given instance of tolerance is often attributed to either a dispositional or a functional change, both types of change can contribute to a given instance of drug tolerance (see Jaffe, 1980; Kalant et al., 1971; Wood & Laverty, 1979). The following two sections briefly review the various mechanisms assumed to be responsible for dispositional and functional drug tolerance.

Dispositional Mechanisms of Tolerance. Dispositional change refers to any instance in which previous exposure to a drug diminishes its efficacy by reducing its concentration at its site of action (Kalant et al., 1971) or by decreasing the duration of time that the drug remains at its site of action (Lê & Khanna,

1989). Dispositional tolerance has been attributed to one of four different mechanisms; to changes in the 1) absorption, 2) distribution, 3) breakdown, or
4) clearance of the drug after repeated administration.

1. Absorption. Before a drug can affect the central nervous system, it must be absorbed from its site of administration into the general circulation. As Lê and Khanna (1989) point out, the importance of drug absorption to the development of tolerance is dependent upon the route of drug administration; drug absorption is relatively unimportant when a drug is administered by microinjection directly into the brain (because the drug avoids the general circulation) or intravenously (because the drug is administered directly into the general circulation), more important when a drug is administered intraperitoneally (because the drug must be absorbed by the vascularization in the peritoneal cavity to enter the general circulation), and very important when a drug is administered orally (because the absorption of the drug into the general circulation is influenced by gastric emptying and the vascularization of the gut and small intestine).

2. Distribution. Once a drug has entered the general circulation, it may be distributed to a variety of fluid and tissue "compartments" before gaining access to its site of action. For example, ethanol is widely distributed throughout the entire body water; the distribution of other drugs may be restricted by the degree to which they bind to plasma proteins

(e.g., albumin) or are absorbed by various body tissues (e.g., adipose tissue or bone). Changes in the distribution of a drug may greatly affect the concentration at its site of action, and thus its effect on the central nervous system.

3. Breakdown. The most well-known mechanism of dispositional tolerance is the increase in drug breakdown that can occur when a drug is repeatedly administered. This increase in drug metabolism is often the result of an induction of hepatic enzymes that are capable of metabolizing a wide variety of drugs. As a result, in many instances the induction of hepatic enzymes by repeated exposure to one drug will result in the development of tolerance to that drug's effects and cross-tolerance to the effects of other drugs that are also susceptible to breakdown by these enzymes (see Kalant, 1989).

4. Clearance. The rate of a drug's elimination from the body will obviously influence the duration and magnitude of a drug's effects. Drugs and/or their metabolic byproducts are usually excreted in urine by the kidney, though significant drug elimination can also occur in the feces via the liver and through the lungs and sweat glands.

Functional Mechanisms of Tolerance. Functional tolerance refers to a reduction in the efficacy of a drug that is attributable to a decrease in the sensitivity of the physiological systems affected by the drug rather than to a decrease in the concentration of the drug itself (Jaffe, 1980; Kalant et al., 1971; Lê & Khanna, 1989). That is, functional tolerance is an

adaptation to the effects of a drug on the function of a physiological system rather than to a decrease in its presence per se (see also Kalant et al., 1971; Jaffe, 1980; Kalant, 1989). It is important to keep in mind that the changes underlying the development of functional drug tolerance do not necessarily have to occur at the drug's primary site of action (e.g., tolerance may be mediated by "subsensitive" receptors on postsynaptic neurons that are exposed to an increase in neurotransmitter release that is produced by a drug's effect on presynaptic neurons); this greatly complicates the study of the physiological bases of functional drug tolerance "as much of our effort, at the more basic levels, may be describing phenomena that are not obviously related to the phenomena of tolerance..." (Martin, 1984).

A variety of physiological alterations have been proposed to underlie the development of functional drug tolerance. These include changes in the sensitivity or number of neurotransmitter or drug receptors (e.g., Gallager & Gonsalves, 1988; Rosenberg et al, 1986); changes in the synthesis, release, or reuptake of various neurotransmitters (e.g., Löscher, 1986a; Melchior & Tabakoff, 1981), neuromodulators (e.g., Vollicer & Ullman, 1985), or hormones (e.g., Wood, 1977; Tabakoff & Yanai, 1979); changes in cell membrane composition (e.g., Goldstein, 1983); changes in the activity of secondary messengers necessary for many neurotransmitters to have a postsynaptic effect (e.g., Siggins, 1979); or changes in ion conductances across the neuronal

membrane (e.g., Littleton & Little, 1989; Ross, Garrett, & Cardenas, 1979). In many instances, the development of functional drug tolerance to a single drug effect is likely dependent upon a combination of physiological changes. Furthermore, functional tolerance to different effects of a single drug have been attributed to a variety of distinct and independent physiological changes (e.g., Rosenberg, Chiu, & Teitz, 1986; Teitz & Rosenberg, 1988). Given the number and combination of physiological changes that may underlie a given instance of functional drug tolerance, it is not surprising that few definitive statements can be made about its physiological bases (see Kalant, 1989).

Pharmacologic Drug Tolerance

Conventional research on the phenomenon of drug tolerance has been greatly influenced by the assumption that the administration of a drug is a sufficient impetus for the dispositional and functional changes that underlie the development of tolerance to the drug's effects. More specifically, the assumption has been that drug exposure automatically produces the changes in absorption, distribution, metabolism, or clearance necessary for dispositional tolerance; and that drug exposure inevitably produces the drug effects that are necessary for the physiological adaptations underlying the development of functional tolerance. The implication of this focus is that drug tolerance is a function of a variety of

pharmacologic factors and that factors such as the context in which the drug is administered or the ongoing physiological and behavioral activity of the drug recipient during periods of drug exposure are inconsequential. This pharmacologic view of drug tolerance can be summarized as follows:

DRUG EXPOSURE ---> DISPOSITIONAL CHANGES AND/OR FUNCTIONAL CHANGES ---> DRUG TOLERANCE

Because this view of drug tolerance has guided much of the work in the area, a considerable amount is known about the effect of pharmacologic factors on the development or dissipation of tolerance. For example, it has been shown that the following factors facilitate the development of tolerance: 1) increasing the size of the treatment dose (e.g., Lê, Khanna, & Kalant, 1984); 2) increasing the total number of drug administrations (e.g., Lê, Kalant, & Khanna, 1986); 3) using a shorter rather than longer interdose interval (e.g., Giknis & Damjanov, 1984); 4) using a drug with a long half-life (e.g., Okamato, 1984); 5) using subjects that have previously developed (and then lost) tolerance to the effects of that drug (this is referred to as tolerance carry-over; Kalant et al., 1971); 6) using subjects that have developed tolerance to the effects of a drug with a similar dispositional profile or mechanism of action (this is referred to as cross-tolerance; e.g., Kalant et al., 1971); or 7) using subjects that are particularly sensitive to the drug's

effect (e.g., Khanna, Lê, LeBlanc, & Shah, 1985).

According to the pharmacologic view of drug tolerance, tolerance dissipates as a function of time since the cessation of drug treatment (e.g., Teitz & Rosenberg, 1988). It has also been demonstrated that the dissipation of tolerance to a drug's effects can be facilitated by the administration of pharmacologic agents that antagonize the effects of the drug (e.g., Gallager & Gonsalves, 1988).

Shortcomings of the Pharmacologic View of Drug Tolerance.

The development and dissipation of drug tolerance are obviously influenced by pharmacologic factors. However, in the last two decades it has become increasingly apparent that the development and dissipation of drug tolerance is also influenced by a variety of behavioral and physiological processes not directly related to a drug's administration--factors such as the context of the drug experience, the behavioral tasks facing the subject during the periods of drug exposure, and the activity of the organism's nervous system during the periods of drug exposure (see also Balster, 1984; Goudie & Emmett-Oglesby, 1989). In general, these factors do not play a minor role in the phenomenon of drug tolerance. In many instances, the development or expression of the dispositional and/or functional changes that underlie a particular instance of tolerance are completely dependent upon the physiological or psychological activity of the drug recipient during the periods of drug exposure or the context in which the drug is administered (see Lê & Khanna, 1989;

Demellweek & Goudie, 1983b; Siegel, 1989).

The effect of these behavioral and physiological variables upon the development and dissipation of drug tolerance has been demonstrated by two types of research. The first type of research has focused on the phenomenon of context-dependent or conditioned drug tolerance, in which the expression of tolerance to a drug's effect is greatly influenced by the presence or absence of environmental cues that have become associated with the drug's effects (Baker & Tiffany, 1985; Eikelboom & Stewart, 1982; Paletta & Wagner, 1986; Siegel, 1975; 1977, 1989; Siegel & MacRae, 1984; Solomon, 1977; Wikler, 1948; 1973). The second type of research that has demonstrated the important effect that behavioral and physiological variables can have on the development of drug tolerance has focused upon the behavioral or physiological responses of the drug recipient during periods of drug exposure. It has been repeatedly demonstrated that the development of tolerance to many drug effects is contingent upon the drug recipient engaging in a particular activity during periods of drug exposure--such tolerance has been termed contingent tolerance (Carlton & Wolgin, 1971; see also Wolgin, 1989; Demellweek & Goudie, 1983b; Goudie & Griffith, 1985). The next section of the Introduction provides a brief review of the phenomena of context-specific tolerance; this will be followed by a more extensive review of the contingent tolerance literature.

2. Context-Specific Drug Tolerance

The manifestation of many types of drug tolerance has been shown to be dependent upon the context in which the subjects have previously experienced the drug's effects (see Baker & Tiffany, 1985; Goudie & Demellweek, 1986; Paletta & Wagner, 1986; Siegel, 1978; 1989; Wikler, 1973). In instances of context-dependent tolerance, subjects display considerable tolerance to the effects of a drug if they are tested in the same context in which its effects had been previously experienced; in contrast, the same subjects display little or no tolerance to the drug's effects if the test dose of the drug is experienced in a context with no drug-related history. Such context-specific tolerance has been demonstrated to the effects of: 1) amphetamine (e.g., Poulos & Hinson, 1984); 2) benzodiazepines (e.g., Greeley & Cappell, 1985); 3) caffeine (e.g., Rozin, Reff, Mark, & Schell, 1984); 4) ethanol (e.g., Mansfield & Cunningham, 1980); 5) haloperidol (Poulos and Hinson, 1982); 6) morphine (e.g., Siegel, 1975, 1977); 7) pentobarbital (e.g., Hinson, Poulos, & Cappell, 1982; Siegel, 1988); and 8) scopolamine (e.g., Poulos, Wilkinson, & Cappell, 1981).

According to Siegel (1975; 1989; Siegel & Macrae, 1984), the context specificity of this tolerance is the consequence of Pavlovian conditioning. It is Siegel's view that the context in which a subject repeatedly experiences the drug's effects acts as a conditional stimulus (CS) that becomes associated with the unconditional effects of the drug (the unconditional stimuli or

UCS's). Siegel argues that as this association is strengthened, the context begins to elicit a conditional compensatory response (CCR) that opposes the unconditional effects of the drug and increases in magnitude as the association between the context and the drug's effects strengthens. Because the CCR is expressed only when the drug is administered in the presence of drug-predictive cues, the manifestation of tolerance is context-specific.

Siegel's Pavlovian explanation of context-specific tolerance is supported by several lines of evidence. First, the development of context-specific tolerance is sensitive to a variety of Pavlovian procedures. For example, the development of context-specific tolerance shows a CS preexposure effect; that is, if subjects are repeatedly presented with the context that is to become the CS prior to the regimen of drug exposure, the development of tolerance when the context and the drug's effects are subsequently paired is much slower than it would be if the subjects had no prior experience with the context (Siegel, 1977). Similarly, instances of context-specific tolerance have been found to be sensitive to extinction procedures (Siegel, 1975; Greeley, Lê, Poulos, & Cappell, 1984), partial reinforcement effects (Siegel, 1978; Krank, Hinson, & Siegel, 1984), conditioned inhibition (Siegel, Hinson, & Krank, 1981), and overshadowing (Walter & Riccio, 1984).

The second, and most direct, line of evidence supporting Siegel's Pavlovian theory of context-specific tolerance has been

provided by demonstrations of the conditional compensatory response, the hypothetical construct on which Siegel's theory is based. The administration of a placebo to tolerant subjects in the drug-predictive environment has frequently been reported to elicit a response opposite to the initial effect of the drug. For example, placebo injections in the drug-predictive environment have been shown to elicit hyperalgesia in rats that have developed context-specific tolerance to the analgesic effect of morphine (Krank, Hinson, & Siegel, 1981), hypothermia in rats tolerant to morphine's hyperthermic effect (Siegel, 1978), hyperthermia in rats tolerant to chlordiazepoxide's hypothermic effects (Greeley & Cappell, 1985), or hyperactivity in rats tolerant to ethanol's hypoactive effect (Mansfield & Cunningham, 1980).

Unfortunately, many attempts to demonstrate a conditional compensatory response have been unsuccessful, and Siegel's Pavlovian model of context-specific tolerance has been criticized on this basis by a number of researchers (e.g., Baker & Tiffany, 1985; Goudie & Demellweek, 1986; Goudie & Griffiths, 1985; Shapiro, Dudek, & Rosellini, 1983; Tiffany, Baker, Petrie, & Dahl, 1983). In what is arguably the most well-developed alternative to Siegel's theory of context-specific tolerance, Baker and his colleagues (Baker & Tiffany, 1985; Kesner & Baker, 1981; Kesner & Cook, 1983) have argued that such tolerance can be attributed to a conditioned habituation to the drug's effects (see also Siegel, 1977; Solomon, 1977; Wagner, 1978; 1981, for

earlier versions of this idea). According to this theory of context-specific tolerance, repeated administration of a drug in a particular environment leads to the development of an association between the contextual cues and the drug's effects. As a result of this association, subsequent presentation of the contextual cues leads to the retrieval from long-term memory of a representation of the drug's effects. This "associatively generated priming result[s] in decreased neural processing of the drug stimulus. Such decreased processing of drug stimulus information results in [sic] attenuated behavioral effect and constitutes tolerance." (Baker & Tiffany, 1985, p. 83).

Paletta and Wagner (1986) have proposed a compromise between the positions taken by Baker and Tiffany(1985) and Siegel (e.g., Siegel & MacRae, 1984), in which an environmental stimulus that has become a CS for a drug-effect US will always produce a conditioned habituation to the US, but may also elicit a conditional compensatory response as well. Paletta and Wagner (1986) argue that the critical factor in the development of a conditional compensatory response is the existence of a "compensatory" secondary UCR elicited by the drug (e.g., a biphasic response; for example, hypothermia followed by hyperthermia). Context-dependent tolerance for all drugs involves a conditional habituation (as suggested by Baker & Tiffany, 1985); when the CS overlaps the secondary, "compensatory" portion of the UCR a conditional compensatory response (as suggested by Siegel & MacRae, 1984) will also be

elicited.

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

3. Contingent Drug Tolerance

Introduction.

Contingent drug tolerance is a form of functional tolerance that develops preferentially to a drug's effects on those activities that occur during periods of drug exposure. It is usually demonstrated in terms of the difference in tolerance development observed between the two groups of subjects in what has been termed the before-and-after design (Kumar & Stolerman, 1977). In this design, the subjects in one group (the drug-before group) receive the drug before engaging in a particular activity (the criterion activity) on each tolerance-development trial so that the activity is performed while the subject is under the influence of the drug. The subjects in the second group (the drug-after group) receive the drug after engaging in

the criterion activity. On the test trial, all subjects receive the drug before the performance of the criterion activity so that the drug's effects on it can be assessed. Any evidence of greater tolerance in the drug-before subjects is attributed to the relation between the criterion activity and the period of drug exposure because the subjects in the two groups do not differ in either their exposure to the drug or in their opportunity to engage in the criterion activity (though see Wolgin, 1989).

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

Early Studies of Contingent Tolerance. Newman and Card (1937) were perhaps the first to propose that the behavior of a subject during periods of drug exposure might influence the development of tolerance to the drug's effects; however, it was not until the seminal reports of Schuster, Dockens, and Woods (1966), Chen (1968), and Carlton and Wolgin (1971) that the idea began to attract significant attention.

Schuster et al. (1966) reported that the development of

tolerance to amphetamine's effects on operant responding in rats was dependent on the schedule of reinforcement that was used during the periods of drug exposure; rats developed tolerance to amphetamine's effects on their bar-press behavior only when the drug's effects decreased the rate of positive reinforcement (i.e., decreased delivery of food) or increased the rate of negative reinforcement (i.e., avoidance of shock). Schuster and his colleagues (1966) concluded that "the common physiological mechanisms responsible for drug tolerance cannot be appealed to as an explanation (for the specificity of this differential tolerance; p. 177)" and that "tolerance will develop in those aspects of the organism's behavioral repertoire where the action of the drug is such that it disrupts the organism's behavior in meeting the environmental requirements for reinforcements." (p. 181). Unfortunately, Schuster et al. (1966) failed to include a control group that received the same drug experience without the opportunity to respond during the periods of drug exposure; thus, it was impossible for them to unequivocally conclude that the differential tolerance demonstrated by their subjects was a consequence of responding on a given schedule during periods of amphetamine exposure or rather than simple drug exposure.

Chen (1968) avoided this problem by introducing the before-and-after design to study the effect that performance of a maze task while under the influence of ethanol had on the development of tolerance to ethanol's disruptive effects on the performance

of the task. He trained rats to perform a maze task and then assigned them to one of two groups. The rats in one group received ethanol before running the maze on each tolerance-development trial, whereas the rats in the other group ran the maze before receiving ethanol. Chen found that only those subjects given the opportunity to practice the maze while under the influence of ethanol on the tolerance-development trials subsequently demonstrated tolerance to its disruptive effects, in spite of the fact that the rats in the ethanol-after group had received the same number of ethanol injections and had the same amount of experience with the maze.

Carlton and Wolgin (1971) used the before-and-after design to study the effect that eating during periods of drug exposure have on the development of tolerance to d-amphetamine's anorexigenic effect. They found that rats given an injection of amphetamine shortly before they were given access to a sweet milk solution developed tolerance to the drug's anorexigenic effect within an average of four treatment sessions. In contrast, rats that received the same dose of amphetamine after they had consumed the milk solution showed no sign of tolerance after eight treatment trials. Furthermore, the amphetamine-after group did not display an accelerated rate of tolerance development when they were subsequently switched to an amphetamine-before-milk regimen. Carlton and Wolgin (1971) coined the term <u>contingent tolerance</u> to describe their observation that the development of tolerance to the anorexigenic effect of d-amphetamine in rats is

contingent upon providing the subjects with an opportunity to eat during each period of drug exposure.

An Analogy for Contingent Drug Tolerance. Demonstrations of contingent tolerance all support the idea that the performance of some criterion response during periods of drug exposure can influence the development of tolerance to the drug's effect on that response. The importance of drugged responding in the development of contingent tolerance can best be understood by recalling that functional tolerance develops not to the systemic presence of a drug but to its effects (see Demellweek & Goudie, 1983b; Jaffe, 1980; Kalant, 1985; Kalant et al., 1971; Okamoto, Boisse, Rosenberg, & Rosen, 1978). In many instances, the expression of a drug's effects is a normal consequence of drug exposure; accordingly, the development of tolerance is assumed to be a function of drug exposure. In other instances, however, the expression of a drug's effect is contingent upon, or is facilitated by, the activity of the drug recipient during the periods of drug exposure. In such cases, it is possible to show that the drug effect, rather than the exposure to the drug per se, is the critical factor in the development of tolerance.

Poulos and his colleagues (Poulos & Hinson, 1984; Poulos et al., 1981) illustrated the role of the criterion response in the development of contingent tolerance with an interesting analogy to a well-known perceptual phenomenon. According to these authors, to expect tolerance to develop in the absence of the criterion response is "like expecting adaptation to the effects

of laterally displacing prisms to develop in an organism maintained in the dark. Without an adequate instigating stimulus to provide the basis for perceptual adaptation, none can occur" (Poulos et al., 1981, p. 745). Although their allusion to the "displaced vision" phenomenon is insightful, it requires a slight but significant modification. It is not light per se, but the subject's visual perception of "self-produced movement... with its contingent reafferentation stimulation [that] is the critical factor in compensating for displaced visual images" (Held, 1972, p. 375; see also Rock & Harris, 1972). That is, adaptation to the disruptive effects of visual displacement on visuomotor responding does not occur unless such responding occurs under the influence of the displaced vision. In the same way, tolerance to a drug's effects does not develop unless the effects are expressed. In instances of contingent tolerance, performance of the criterion response during periods of drug exposure facilitates the expression of the drug's effect and thus the development of tolerance to that effect.

Generality of Contingent Tolerance. The activity of the drug recipient during periods of drug exposure has been shown to be an important, if not critical, factor in the development of tolerance to a wide variety of drug effects. This subsection presents representative examples of contingent tolerance to the effects of the following drugs: 1) amphetamine and other psychostimulants; 2) morphine; 3) delta-9-THC; 4) the barbiturates; 5) the benzodiazepines; and 6) ethanol. A more

comprehensive summary of the contingent tolerance literature is available in an excellent recent review by Wolgin (1989).

1. Contingent Tolerance to the Effects of Psychostimulants. Carlton and Wolgin's (1971) seminal report that the development of tolerance to the anorexigenic effects of amphetamine is contingent upon the opportunity to eat during periods of drug exposure has since been confirmed by a number of researchers (e.g., Demellweek & Goudie, 1982; 1983a; Emmett-Oglesby, Spencer, Wood, & Lal, 1984; Poulos et al., 1981). Contingent tolerance also has been demonstrated to the anorexigenic effects of other psychostimulants: cocaine (Woolverton, Kandel, & Schuster, 1978), cathinone (Foltin & Schuster, 1982), methylphenidate (Emmett-Oglesby & Taylor, 1981), and the serotonergic receptor agonist quipazine (Rowland & Carlton, 1983).

The pioneering work of Schuster and his colleagues on the importance of the reinforcement schedule during periods of drugged responding to the development of tolerance to the effects of psychostimulant drugs on operant responding has been widely supported (e.g., Campbell & Seiden, 1973; Emmett-Oglesby et al., 1984; Smith, 1986a; see Wolgin, 1989). In a particularly interesting paper, Smith (1986a) found that rats trained to bar press on a schedule of reinforcement that alternated between a random-ratio (RR) schedule and a differential-reinforcement-for-low-rates-of-responding (DRL) schedule developed tolerance to the drug's effect only on the RR portion of the schedule. When these rats were given additional trials in which only the DRL component

of the reinforcement schedule was used, they rapidly developed tolerance to amphetamine's effects on their responding but this tolerance disappeared as soon as the RR component of the reinforcement schedule was reintroduced. Smith (1986a) suggested that this pattern of results could be accounted for if the development of tolerance was a response to amphetamine's effects on the "global" density of reinforcement and not to the drug's effects on each component of the reinforcement schedule. Because the loss of reinforcement was much greater during the RR component than the DRL component of the schedule, the rats were more likely to demonstrate tolerance during the RR component than the DRL component when both were active.

2. Contingent Tolerance to the Effects of Morphine. Mitchell and his colleagues (e.g., Kayan, Woods, & Mitchell, 1969) were the first to report that the development of tolerance to morphine's analgesic effect was facilitated if rats received nociceptive stimulation (i.e., a hotplate test of nociception) during periods of drug exposure (see also Advokat, 1989; Milne, Gamble, & Holford, 1989; Moore, 1983). A similar effect has been demonstrated in human subjects (Ferguson & Mitchell, 1969). Advokat's (1989) study is particularly noteworthy. She found that nociceptive stimulation during periods of morphine exposure is critical to the development of tolerance to the drug's analgesic effects in spinally transected, but not intact, rats. Based upon these observations, Advokat (1989) suggested that the physiological changes underlying the development of contingent

tolerance to morphine's analgesic effects are localized to spinal circuits.

Because none of these studies used the before-and-after design, they do not provide unequivocal evidence that nociceptive stimulation during periods of drug exposure is an important factor in the development of such tolerance to morphine's analgesic effect. However, a before-and-after design has been used to show that the drugged responding plays an important role in the development of tolerance to the disruptive effects of morphine on operant responding in the rat (Sannerud & Young, 1986).

3. Contingent Tolerance to the Effects of Barbiturates.

Contingent tolerance has been demonstrated to the disruptive effects of barbiturates on operant behavior (e.g., Branch, 1983; Harris & Snell, 1980; Tang & Falk, 1978) and rotorod performance (Commissaris & Rech, 1981). Tang and Falk's (1978) demonstration is particularly noteworthy. They assessed the development of contingent tolerance to phenobarbital's effects on operant responding in terms of the dose-response curves obtained for both the drug-before and the drug-after groups prior to and after the tolerance-development phase. They found little evidence of contingent tolerance when low test doses of the drug were administered; however, as the test dose was increased the drug-before rats demonstrated progressively greater tolerance to phenobarbital's effects than the rats from the drug-after group.

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

Contingent tolerance has been demonstrated to the disruptive effects of delta-9-THC on bar-press behavior in the monkey (Carder & Olson, 1973; Elsmore, 1972), on bar-press and avoidance behavior in the rat (Manning, 1976a,b), and operant responding in the pigeon (Smith, 1986b). Manning's (1976a,b) experiments are noteworthy in that he found that the development of contingent tolerance to delta-9-THC's effect on operant responding was not influenced by prior drug history; rats that had previously received the drug after performing the criterion activity developed tolerance no faster than drug-naive controls when both groups of rats received the drug before performing the task.

5. Contingent Tolerance to the Effects of Benzodiazepines. There have been only two reported attempts to demonstrate contingent tolerance to the effects of benzodiazepines. Griffiths and Goudie (1987) found that tolerance readily developed to the effects of the short-acting benzodiazepine midazolam on a food-reinforced bar-press task; however, rats that received midazolam before the opportunity to bar-press for food did not develop tolerance any faster, or to any greater extent, than rats that did not receive the drug until after performing the operant task. Griffiths and Goudie (1987) concluded that "Tolerance to midazolam cannot therefore be explained in terms of learned strategies acquitted as a result of drug-induced loss of rewarding stimuli." (p. 201).

In contrast, Herberg and Montgomery (1987) found that the

development of tolerance to the depressant, but not the facilitatory, effects of chlordiazepoxide on intracranial self-stimulation was contingent upon their subjects having the opportunity to self-stimulate during periods of drug exposure (see also Herberg & Williams, 1983). Herberg and Montgomery (1987; 1988) have suggested that the inconsistency between their data and those reported by Griffiths and Goudie (1987) may be due to the fact that chlordiazepoxide has a much longer half-life than midazolam, thereby allowing more time for the acquisition of strategies that would reduce the disruptive effects of the drug.

6. Contingent Tolerance to the Effects of Ethanol. As described earlier in this section, Chen (1968) provided the first report of contingent tolerance to ethanol's effects. Since then, contingent tolerance has been demonstrated to a variety of ethanol's effects. For example, contingent tolerance has been demonstrated to the effect of ethanol on treadmill running (LeBlanc, Gibbins, & Kalant, 1973; 1975; Wenger, Tiffany, Bombardier, Nicholls, & Woods, 1981) and operant responding (Chen, 1979; Wiggell & Overstreet, 1984) and to the ethanol-induced acceleration in the decay of postsynaptic potentiation in the abdominal ganglia of the marine mollusc Aplysia (Traynor, Schlapfer, & Barondes, 1980). Contingent tolerance has also been demonstrated to ethanol's analgesic effect, using the tail-flick test of analgesia (Jørgenson & Hole, 1984; Jørgenson, Berge, & Hole, 1985; Jørgenson, Farmer, & Hole, 1986), and to ethanol's hypothermic effect (Alkana, Finn, & Malcolm, 1982). This latter

study is noteworthy because of the novel method used to manipulate alcohol's hypothermic effect. Alkana and his colleagues found that mice that received an injection of ethanol and were then immediately warmed with microwave radiation, so that ethanol's hypothermic effect could not be experienced, displayed no tolerance on the test trial. In contrast, mice that were injected with ethanol and left at room temperature displayed a substantial amount of tolerance to ethanol's hypothermic effects (N.B., Lê, Kalant, & Khanna [1986a] found that warming the environment in which ethanol's effects are experienced only slowed, rather than prevented, the development of tolerance to its hypothermic effects). Finally, Pinel and his colleagues have reported that the development of tolerance to ethanol's disruptive effects on sexual behavior in rats is contingent upon the rodents having the opportunity to engage in sexual behavior while they are intoxicated (Pinel, Pfaus, & Christensen, submitted).

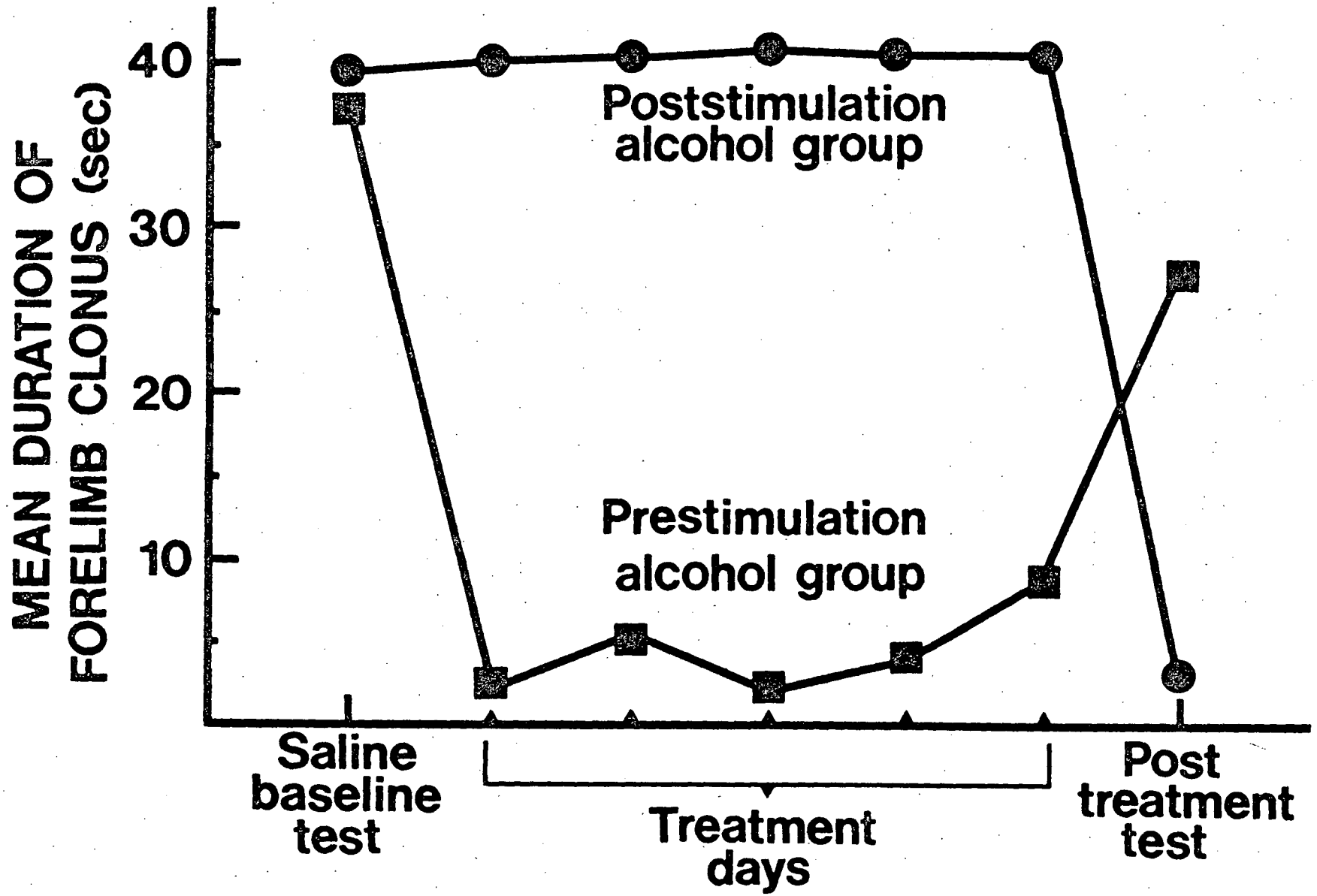
In the last seven years, Pinel and his colleagues have shown that the development of tolerance to ethanol's anticonvulsant effect on convulsions elicited in amygdala-kindled rats is contingent upon the administration of convulsive stimulation during periods of ethanol exposure. Because this work provided the basis for the present thesis, it is described in detail in the next section.

4. Contingent Tolerance to Ethanol's Anticonvulsant Effect

In their original report, Pinel and his colleagues (1983) used a before-and-after design to examine the effect that convulsive stimulation administered during periods of ethanol exposure had on the development of tolerance to ethanol's anticonvulsant effect. After establishing a bidaily (once every 48 hr) convulsive stimulation baseline, kindled rats were assigned to either an ethanol-before-stimulation group or an ethanol-after-stimulation group. The subjects in both groups were then stimulated six more times on the bidaily schedule. The ethanol-before-stimulation rats received ethanol (4.5 g/kg, by intubation, in a 30% volume/volume solution) 1.5 hr before each stimulation and a comparable volume of isotonic saline 1.5 hr afterwards. The ethanol-after-stimulation rats received the same intubations but in the reverse order (i.e., the saline before each stimulation and the ethanol after). On the test trial, the rats from both treatment groups were challenged with an injection of ethanol (1.5 g/kg, IP, in a 25% volume/volume solution) 1.5 hr before the test stimulation.

As is evident in Figure 1, the rats in the ethanol-before-stimulation group displayed substantial tolerance to ethanol's anticonvulsant effect on the test trial. In contrast, there was no evidence of tolerance in any of the subjects from the ethanol-after group. A subsequent analysis of the blood ethanol level for each rat immediately after testing revealed no significant difference between the two groups.

FIGURE 1. The effect of the response contingency on the development of tolerance to ethanol's anticonvulsant effect. During the treatment phase, ethanol (4.5 g/kg) was intubated at 48-hr intervals, either before or after convulsive stimulation. On the test trial, the rats that had received ethanol before stimulation on the treatment days (the ethanol-before-stimulation group) demonstrated substantial tolerance to the anticonvulsant effect of the test dose of ethanol (1.5 g/kg, IP), whereas there was no evidence of tolerance in the rats that had received ethanol after each convulsive stimulation on the treatment days (the ethanol-after-stimulation group). (From Pinel et al., 1983).


MEAN DURATION OF FORELIMB CLONUS (sec)
40
30
20
10
Poststimulation alcohol group
Prestimulation alcohol group
Saline baseline test
Treatment days
Post treatment test

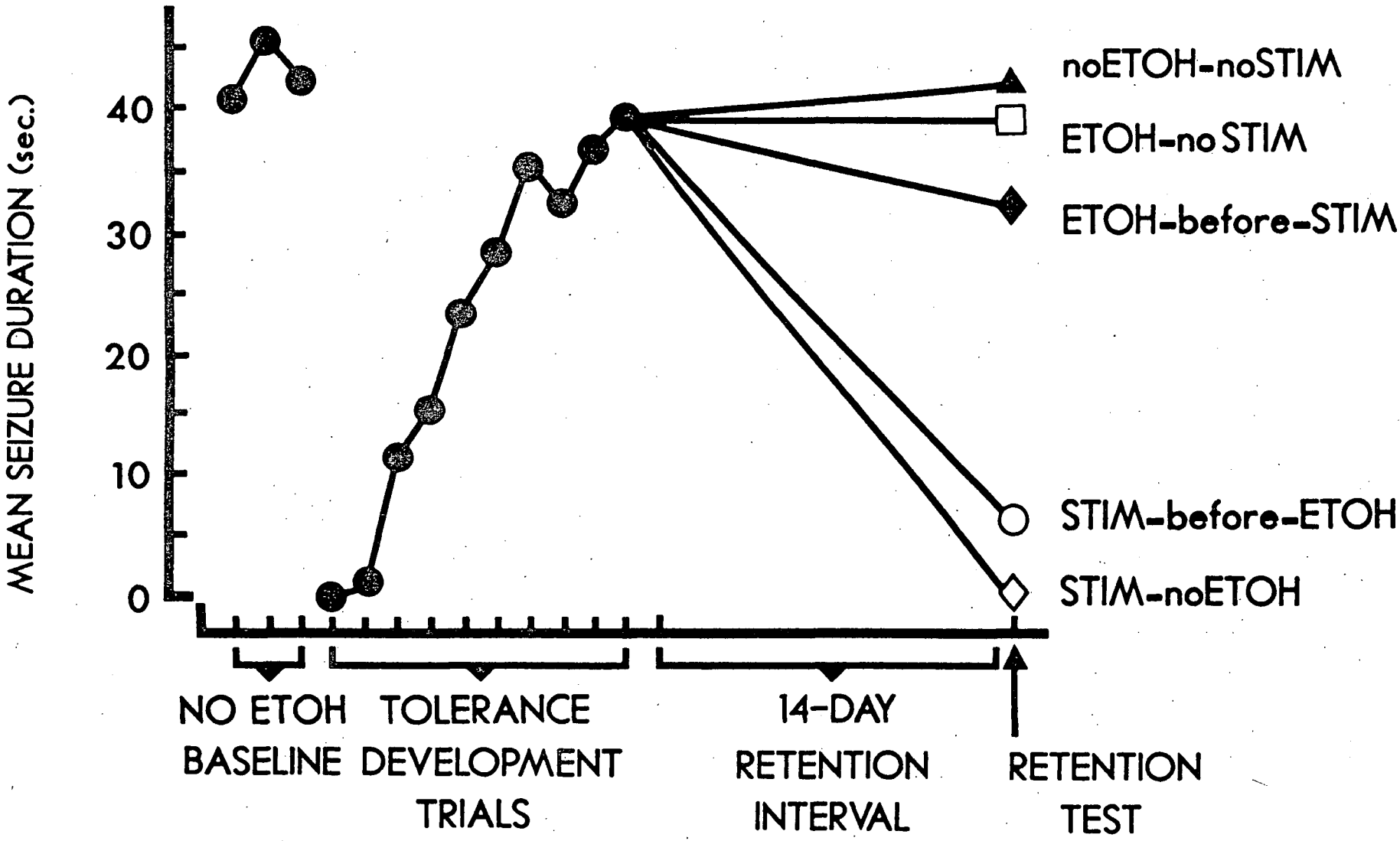
Aside from the sizable effect that convulsive stimulation had on the development of tolerance to ethanol's anticonvulsant effect, perhaps the most notable result of Pinel et al.'s (1983) experiment was the lack of any evidence of tolerance in the ethanol-after-stimulation group: ethanol exposure alone produced no detectable tolerance. In a follow-up series of experiments, Pinel et al. (1985) extended this finding by showing that kindled rats that received ethanol 1 hr after each bidaily stimulation failed to develop tolerance to ethanol's anticonvulsant effect even when the treatment dose was as high as 5 g/kg (by gavage), or as many as 20 tolerance-development trials were conducted prior to the test trial. Mana, Lê, Kalant, and Pinel (in preparation) subsequently demonstrated that kindled rats that received a total of 20 ethanol intubations (5 g/kg), on a daily rather than bidaily basis, developed tolerance to ethanol's anticonvulsant effect in the absence of concurrent convulsive stimulation. There is no doubt, however, that the development of tolerance to the anticonvulsant effects of ethanol is greatly facilitated by the administration of convulsive stimulation during periods of ethanol exposure.

Pinel and Puttaswamaiah (1985) demonstrated that contingent tolerance to ethanol's anticonvulsant effect is not a form of conditional or context-dependent tolerance. There was no evidence that contingent tolerance to ethanol's anticonvulsant effect is context specific; the tolerance that the kindled rats displayed in an environment that had never been paired with

ethanol was just as robust as that displayed by the rats in the environment that was consistently paired with the ethanol injections during the tolerance-development phase. Furthermore, they found no evidence of a conditional compensatory response when tolerant rats received a saline injection in a drug-predictive environment, and preexposure to the contextual cues associated with ethanol administration had no effect on tolerance development. And Mana and Pinel (unpublished observations) tested the idea that the cue properties of the convulsive stimulation itself could serve as a CS predictive of ethanol's anticonvulsant effect. They found that varying the number of stimulations that kindled rats received (i.e., the degree of CS preexposure) prior to the start of the tolerance-development phase had no effect on the rate or magnitude of tolerance that developed.

The fact that convulsive stimulation played such a critical role in the development of tolerance to ethanol's anticonvulsant effect led Mana and Pinel (1987) to investigate the role of convulsive stimulation in the dissipation of contingent tolerance to ethanol's anticonvulsant effect. Drug withdrawal is generally considered to be the necessary and sufficient condition for the dissipation of tolerance to a drug's effects. In contrast, Mana and Pinel (1987) found that ethanol withdrawal was neither necessary nor sufficient for the dissipation of tolerance to ethanol's anticonvulsant effect; instead, the administration of convulsive stimulation in the absence of ethanol was the critical

Figure 2. Contingent dissipation of tolerance to ethanol's anticonvulsant effects on kindled convulsions. Ethanol withdrawal had no effect on the dissipation of tolerance, as the rats in the No Ethanol-No Stim group demonstrated no loss of tolerance even though they were not administered ethanol during the retention interval. Furthermore, the administration of ethanol was not a necessary condition for the maintenance of tolerance, as the rats in the Ethanol-After-Stimulation group lost their tolerance even though they continued to receive ethanol on the same schedule of administration associated with the development of tolerance. The critical factor in the dissipation of tolerance to ethanol anticonvulsant effects was the administration of convulsive stimulation in the absence of ethanol. (from Mana & Pinel, 1987).


MEAN SEIZURE DURATION (sec.)
40
30
20
10
0
noETOH-noSTIM
ETOH-no STIM
ETOH-before-STIM
STIM-before-ETOH
STIM-noETOH
NO ETOH BASELINE
TOLERANCE DEVELOPMENT TRIALS
14-DAY RETENTION INTERVAL
RETENTION TEST

factor in the dissipation of tolerance. Kindled rats were rendered tolerant to ethanol's anticonvulsant effect in the usual fashion (i.e., bidaily ethanol injections (1.5 g/kg, IP), each followed 1 hr later by a convulsive amygdala stimulation. They were then assigned to one of six different treatment groups; the rats in each group were treated differently during the ensuing 14-day retention interval. The rats in two groups received an ethanol injection either 1 hr before or 1 hr after each of six bidaily stimulations. The rats in two other groups were treated in the same manner except that saline rather that ethanol was injected. The rats in the fifth group received the six bidaily ethanol injections but no convulsive stimulations, whereas those in the sixth and final group received neither the bidaily stimulations nor the ethanol injections during the retention interval. A single tolerance test trial was administered to all subjects at the end of the 14-day retention interval. This test was identical to the tolerance-development trials; all subjects were stimulated 1 hr after receiving the standard 1.5 g/kg (IP) ethanol test injection.

It is clear in Figure 2 that the dissipation of tolerance to ethanol's anticonvulsant effect over the 14-day retention interval was greatly influenced by the treatment received by the rats during this period. In three of the groups--the saline-before-stimulation and saline-after-stimulation groups (combined in Figure 2), and the ethanol-after-stimulation group--tolerance to ethanol's anticonvulsant effect had completely disappeared

following the retention interval. In contrast, the magnitude of tolerance in the other three groups--the no-ethanol-no-stimulation group, the ethanol-before-stimulation group, and the ethanol-no-stimulation group--did not decline. These results clearly show the inadequacy of the view that the dissipation of ethanol tolerance is a result of the discontinuation of ethanol exposure: The cessation of ethanol exposure did not lead to a decline in tolerance in the rats from the no-ethanol-no-stimulation group, and continued exposure to ethanol did not maintain the tolerance in the rats from the ethanol-after-stimulation group. Instead, the key factor in the dissipation of tolerance to ethanol's anticonvulsant effect appeared to be the administration of convulsive stimulation in the absence of ethanol. Tolerance did not decline at all in the two groups that did not receive any stimulations during the retention interval or in the group receiving stimulations following each ethanol injection, but it dissipated completely in groups stimulated in the absence of ethanol, even when ethanol was still administered after each bidaily stimulation.

The important role that convulsive stimulation has on the dissipation of tolerance to ethanol's anticonvulsant effect can be easily understood if one returns to the prism analogy that was introduced earlier in this Introduction to illustrate the role that convulsive stimulation has on the development of tolerance to ethanol's anticonvulsant effect. Just as subjects that have adapted to the effects of vision-displacing prisms must

experience the effects of their removal for the adaptation to dissipate (see Rock, 1966), so too rats tolerant to the anticonvulsant effects of ethanol must experience seizures in the absence of ethanol for tolerance to dissipate (see Poulos & Hinson, 1984, and Poulos, Wilkinson, & Cappell, 1981, for further evidence that the dissipation of contingent tolerance is influenced by the performance of the criterion response in an undrugged state).

Pinel, Kim, Paul, and Mana (1989) recently demonstrated that the development of cross-tolerance between ethanol and pentobarbital's anticonvulsant effects is also contingent upon the administration of convulsive stimulation during periods of drug exposure. Only rats exposed to pentobarbital on a bidaily drug-before-stimulation treatment regimen subsequently displayed cross tolerance to the anticonvulsant effect of ethanol; pentobarbital-after-stimulation rats displayed no cross tolerance. And the same proved true for the transfer of tolerance in the other direction: Only rats from the ethanol-before-stimulation group displayed cross-tolerance to pentobarbital's anticonvulsant effect.

Although there is now strong evidence that the relation between convulsive stimulation and drug exposure has a substantial effect on development and dissipation of tolerance to ethanol's anticonvulsant effect, there are no published reports suggesting that convulsive stimulation, or any type of seizure activity in the brain, plays a role in the development or

dissipation of tolerance to the effects of clinically relevant antiepileptic drugs. Instead, the research in this area has been predicated on the traditional idea that the development and dissipation of tolerance is strictly a function of pharmacological variables. Because one purpose of this thesis is to compare contingent and pharmacologic tolerance to the anticonvulsant drug effects, the next section briefly reviews the existing literature on pharmacologic tolerance to the anticonvulsant effects of antiepileptic drugs. The first subsection describes the paradigms that have traditionally been used to study the development of tolerance to anticonvulsant drug effects; the next three subsections focus on reports of tolerance to the drugs studied in this thesis: carbamazepine, diazepam, and sodium valproate, respectively.

5. Pharmacologic Tolerance to the Anticonvulsant Effects of Antiepileptic Drugs

Although anecdotal evidence that tolerance develops to the anticonvulsant effects of antiepileptic drugs has existed almost since their introduction as a treatment for the epilepsies in the early 1900's (e.g., phenobarbital; Hauptmann, 1912, cited in Frey, 1985), there was little clear experimental evidence of this phenomenon until Frey and Kampmann published their landmark study of tolerance to the anticonvulsant effects of phenobarbital and phenytoin in 1965. Since then, there have been numerous laboratory demonstrations of tolerance to the anticonvulsant

effects of clinically prescribed antiepileptic drugs.

In contrast, clinical support for the idea that tolerance develops to the anticonvulsant effects of antiepileptic drugs in human epileptics remains equivocal (see Frey, 1987). This apparent inconsistency can be attributed to at least three factors. First, it is routine therapeutic procedure for physicians to increase the dosage of an antiepileptic drug if it does not provide adequate protection once steady plasma levels have been reached (e.g., see Eadie, 1985); thus, the development of tolerance to an antiepileptic drug's anticonvulsant effect may be mistakenly attributed to an inadequate initial treatment dose (see Butler, Mahaffee, & Waddell, 1954 for a prescient discussion of this problem; see also Frey, 1987). Second, ethical considerations restrict the scope of clinical investigations of the development of tolerance to the anticonvulsant effects of antiepileptic drugs. Third, the development of tolerance to the anticonvulsant effect of one drug may often be obscured by the presence of one or more other antiepileptic drugs (see Koella & Meinardi, 1986a)--polypharmacy (the concurrent administration of more than one type of drug) is still the rule rather than the exception in the clinical treatment of epilepsy (Koella & Meinardi, 1986a).

In spite of these difficulties, it is now generally accepted that tolerance does develop to the effects of most antiepileptic drugs in clinical settings (see Frey, 1987). Accordingly, the next four subsections review the evidence that tolerance develops

to the anticonvulsant effects of antiepileptic drugs. The first subsection describes the two types of experimental convlsions that have traditionally been used to study the development of tolerance to anticonvulsant drug effects: maximal electroshock convulsions and pentylenetetrazol convulsions. The final three subsections review both experimental and clinical reports of tolerance to the anticonvulsant effects of the three antiepileptic drugs that are the focus of this dissertation: 1) carbamazepine (CBZ); 2) diazepam (DZP); and 3) sodium valproate (VPA). Each subsection includes a brief description of the drug's history, pharmacologic profile, spectrum of anticonvulsant activity, and history of tolerance.

Traditional Convulsion Paradigms

Maximal Electroshock Convulsions Maximal electroshock (MES) convulsions are commonly used in drug-development trials to assess the anticonvulsant efficacy of drugs (Koella, 1985), as well as to assess the development of tolerance to anticonvulsant drug effects. Although the details of the procedure may vary, MES convulsions are usually studied in rats or mice, with the electrical stimulation (either 60 Hz, alternating sine-wave current for about 0.2 sec, or 6 Hz, pulsating direct current for about 3 sec) administered by ear clips or corneal electrodes (Koella, 1985). Maximal electroshock convulsions are produced by administration of high-intensity stimulation (e.g., in rats, 60 Hz alternating current at 150 mA for 3 sec); they last approximately 20 sec to 25 sec and involve tonic flexion,

followed by tonic extension of all four limbs, and then a period of clonus involving all four limbs (Koella, 1985). Maximal electroshock convulsions are the model of choice in the assessment of drug efficacy against primarily generalized and complex-partial epilepsy (Swinyard, 1980).

Pentylenetetrazol Convulsions Pentylenetetrazol (PTZ) is probably used more often than any other chemical convulsant to assess the development of tolerance to anticonvulsant drug effects. Pentylenetetrazol is usually administered intraventricularly or subcutaneously; slow infusion by the IV route is frequently used when the threshold dose is of interest (Koella, 1985). Convulsions usually begin 60 sec to 210 sec after the administration of PTZ; they generally involve several whole-body clonic convulsions which last from 2 min to 3 minutes. The clonic convulsions are often followed by a tonic convulsion; animals exhibiting this tonic phase usually die. Drugs effective at reducing PTZ convulsions are usually also effective against petit mal and absence seizures in humans (Swinyard, 1980).

Tolerance to the Anticonvulsant Effects of Carbamazepine

History The synthesis of CBZ in 1957 by the laboratories of J.R. Geigy AG was part of a long-term study of the anticonvulsant effects of various iminodibenzyl compounds. Experimental and clinical trials proceeded almost in tandem, with the first reports of its anticonvulsant efficacy in both laboratory animals and humans being published in 1963 (see Schmutz, 1985).

Approved in 1974 for use in the United States as a treatment for epilepsy (see Suria & Killam, 1980), CBZ has become the drug of choice for the treatment of partial and generalized tonic-clonic seizures in both adults and children (see Engel, 1989; Porter, 1986). It is also used in the treatment of trigeminal neuralgia (Rall & Schleifer, 1980).

Pharmacologic Profile CBZ is the only known anticonvulsant possessing a tricyclic structure, more similar to that of the neuroleptic chlorpromazine and the antidepressant imipramine than to other antiepileptic drugs (see Schmutz, 1985). CBZ is insoluble in water but soluble in solvents such as alcohol, acetone, and propylene glycol (Schmutz, 1985).

The pharmacokinetics of CBZ have been well established. Peak plasma levels in the rat are reached within 45 min of IP injection (Moreselli, 1975; Morton, 1984); absorption after oral administration tends to be slower. CBZ is evenly distributed throughout the body; there is no preferential affinity for any particular organs or tissue (Faigle, Brechbüler, Feldman, & Richter, 1976). The metabolism of CBZ is qualitatively similar in rats and man (see Schmutz, 1985); notably, the product of the primary metabolic pathway for CBZ is an epoxy metabolite with significant anticonvulsant properties of its own (Rall & Schleifer, 1980). The metabolism of CBZ is accelerated during chronic exposure to the drug (metabolic tolerance) by the autoinduction of hepatic enzymes (Faigle et al., 1976; see also Morselli, Bossi, & Gerna, 1976, for similar results in human

epileptic patients); thus, it is often necessary to increase the treatment dose several times to maintain an effective plasma concentration.

The mechanism of action for CBZ's anticonvulsant effect is unknown. It is generally agreed that CBZ's anticonvulsant effects are not mediated by a change in the function of the inhibitory neurotransmitter GABA; at therapeutic levels, CBZ does not appear to alter the release, reuptake, or synthesis of GABA in the brain (see Schmutz, 1985). Similarly, dopaminergic, noradrenergic, and serotonergic systems in the CNS do not seem to play a role in CBZ's anticonvulsant effect (see Schmutz, 1985). CBZ has been shown to selectively increase the levels of acetylcholine in the striatum, but not in the hippocampus, diencephalon, mesencephalon, or cerebellum (Consolo, Bianchi, & Ladinski, 1976). The fact that many convulsants increase the release and decrease the brain concentration of acetylcholine led Consolo et al. (1976) to suggest that CBZ's anticonvulsant effect may be mediated by a cholinergic mechanism. It has also been suggested that CBZ's anticonvulsant effect might be due to its partial agonist action at adenosine receptors (Schmutz, 1985; though see Marangos, Post, Patel, Zander, Parmer, & Weiss, 1983), or by a reduction in sodium or potassium currents (Schauf, Davis, & Marder, 1974) or synaptosomal potassium-mediated calcium uptake (e.g., Ferendelli & McQueen, 1982).

Anticonvulsant Activity CBZ has been shown to reduce the severity of a wide variety of experimental convulsions, including

both maximal and minimal electroshock convulsions (Julien & Hollister, 1975; Koella, Levin, & Baltzer, 1976); pentylenetetrazol convulsions (Koella, Levin, & Baltzer, 1976); audiogenic convulsions (Consroe, Kudray, & Schmitz, 1980); kindled convulsions (Weiss & Post, 1987); penicillin-focus convulsions (Julien & Hollister, 1975); and photic convulsions in the baboon Papio papio (Killam, 1976). In clinical practice, CBZ is used in the treatment of complex partial and secondarily generalized epilepsy (Hönack & Löscher, 1989; Schmutz, 1985; Engel, 1989).

Tolerance to CBZ's Anticonvulsant Effects Morselli's (1975) comment that CBZ's "anticonvulsant effect is reduced" in rats that demonstrate an acceleration of CBZ metabolism following chronic exposure may be the first report of tolerance to CBZ's anticonvulsant effect. However, because Morselli (1975) failed to provide any data to support this claim, Farghali-Hassan et al. (1976) are credited with the first conclusive demonstration that tolerance develops to the anticonvulsant effect of CBZ. Farghali-Hassan et al (1976) found that tolerance developed to CBZ's (25 mg/kg, IV) anticonvulsant effect on maximal electroshock convulsions following twice-daily injections of the drug for 12 days. They attributed the development of tolerance to an increase in the rate of metabolism of CBZ, with a corresponding decrease in brain levels of the drug. Similar results were subsequently reported by Masuda, Utsui, Shiraishi, Karasawa, Yoshida, and Shimizui (1979), who also found that the

development of tolerance to CBZ's anticonvulsant effect (50 mg/kg, perioral [PO]) on maximal electroshock convulsions was accompanied by an increase in the breakdown of the drug.

Masuda and his colleagues (1979) also found evidence of a functional component to the tolerance that developed to CBZ's anticonvulsant effect; rats that had received chronic exposure to CBZ were less sensitive to its anticonvulsant effects than were drug-naive control rats displaying an identical plasma concentration of the drug. Similarly, Hönack and Löscher (1989) suggested that the development of tolerance to the anticonvulsant effect of CBZ (30 mg/kg, IP, administered three times per day for 2 weeks) on kindled convulsions in the rat is attributable, in part, to a functional mechanism because of their observation that tolerance developed at significantly different rates to different effects of the drug.

Given the general agreement about the prevalence of dispositional tolerance to CBZ's effects, it is somewhat surprising that Baltzer, Baud, Degen, and Koella (1980) observed no evidence of tolerance to the anticonvulsant effect of CBZ (at daily doses of 6, 10, or 18 mg/kg, PO) on maximal electroshock convulsions, even after 28 daily treatment trials (see also Schmutz, David, Grewal, Bernasconi, & Baltzer, 1986). This discrepancy might be attributable to the fact that Baltzer et al. (1980) administered a relatively low treatment dose to their subjects (the largest dose was 18 mg/kg/day), whereas relatively large treatment doses were administered by Farghali-Hassan et al.

(1976; 50 mg/kg/day), Masuda et al. (1979; 50 mg/kg/day) and Hönack and Löscher (1989; 90 mg/kg/day).

Tolerance to the Anticonvulsant Effect of Diazepam

History Although the first benzodiazepine compounds were synthesized in the early 1930s by Sternbach (see Killam & Suria, 1980), DZP was not approved for clinical use until 1963 (Killam & Suria, 1980). DZP is effective against a wide variety of epileptic seizures; however, it is not used as a primary form of treatment for epilepsy except for certain forms of myoclonic seizures and for status epilepticus. This lack of acceptance is primarily due to the fact that tolerance rapidly develops to the anticonvulsant effect of this drug (see Frey, 1987, Haigh & Feely, 1988) and to the fact that stable therapeutic levels of DZP are difficult to maintain during chronic administration of the drug (Engel, 1989).

Pharmacologic Profile DZP is insoluble in water but easily dissolves in solvents such as diethyl ether, propylene glycol, or ethanol. Its absorption is rapid, with peak plasma levels being reached within 12 min of IP administration in rats (Morton, 1984). DZP is widely distributed through the body in spite of considerable protein binding (Killam & Suria, 1980), although it accumulates preferentially in the liver, lungs, and adipose tissue (Caccia & Garattini, 1985). DZP easily crosses the blood-brain barrier (Killam & Suria, 1980); once in the brain, it accumulates first in cortical gray matter and then is

redistributed to white matter (Harvey, 1980).

Chronic exposure to DZP does not seem to alter its absorption, distribution, or elimination rates (Kaplan & Jack, 1981). DZP is largely eliminated by the body following biotransformation by hepatic enzymes (Caccia & Garratini, 1985). Chronic exposure to DZP does not appear to accelerate the activity of these hepatic enzymes (Killam & Suria, 1980; though see Linnoila, Korttila, & Mattila, 1975, cited in Harvey, 1980); thus, an increase in metabolism is not likely to contribute to the development of tolerance to its effects. Nordiazepam, one of DZP's major metabolites, possesses significant effects of its own; Killam & Suria (1980) reported that nordiazepam is actually more potent than the parent compound at antagonizing both maximal electroshock and pentylenetetrazol-induced convulsions in mice.

It is generally agreed that the majority of DZP's central effects are mediated at the GABA-benzodiazepine-chloride ionophore complex (Bruun-Meyer, 1987). DZP binds to a high-affinity, saturable, stereospecific receptor (Möhler & Okada, 1977; Squires & Braestrup, 1977) that is usually associated with the low-affinity, GABA-A receptor (Haefly, 1989); GABA and DZP allosterically modulate each others receptors so that the presence of one enhances the binding of the other (see Martin, 1987). Functionally, DZP acts at the GABA-benzodiazepine-chloride ionophore complex to increase the frequency of opening of the chloride channel when GABA is also bound to its receptor (Martin, 1987); the net effect of this increase in chloride

conductance is a decrease in neuronal excitability (Haefly, 1989). In addition, Phillis and O'Regan (1988) have provided compelling evidence that some of the actions of the benzodiazepines, including DZP, may be attributable to their inhibitory effect on the reuptake of adenosine, a putative inhibitory neuromodulator (e.g., Nicoll, Malenka, & Kauer, 1990).

Anticonvulsant Activity Benzodiazepines are among the most potent anticonvulsants, effective against a wide variety of experimental convulsions as well as various forms of human epilepsy (see Haefly, Pieri, Polc, & Schaffner, 1981; Caccia & Garattini, 1985). DZP attenuates maximal electroshock convulsions (e.g., Baltzer et al., 1980); bicuculline- and penetylenetetrazol-induced convulsions (e.g., Matthews & McCafferty, 1979); photically induced convulsions in the baboon Papio papio (e.g., Killam, Matsuzaki, & Killam, 1973); kindled convulsions (e.g., Albright & Burnham, 1980); picrotoxin-induced convulsions (e.g., Jenner, Marsden, Pratt, & Reynolds, 1979); and penicillin-induced convulsions (e.g., Stark, Edmonds, & Keesling, 1974) (see Haefly et al., 1981, for a complete review). In general, larger doses of DZP are needed to attenuate electroshock convulsions than are needed to attenuate the effects of chemical convulsants such as pentylenetetrazol, bicuculline, or strychnine (e.g., Haefly et al., 1981).

Tolerance to DZP's Anticonvulsant Effect There are more reports of tolerance to the anticonvulsant effects of the benzodiazepines, including DZP, than to any other antiepileptic

drug. Although anecdotal reports existed earlier, Killam, Matsuzaki, and Killam (1973) were the first to unequivocally demonstrate the development of tolerance to the anticonvulsant effect of DZP (0.2-0.4 mg/kg, SC, administered once per day for up to 16 weeks). Perhaps the most interesting aspect of their data was the finding that tolerance was less likely to develop to the anticonvulsant effects of DZP on photically-induced convulsions in Papio if a relatively high treatment dose (0.5 mg/kg per day) was administered from the start of the treatment regimen; when Killam et al. (1973) followed the conventional clinical wisdom of starting with a "threshold" dose of DZP that was just large enough to suppress convulsive activity, tolerance rapidly developed to the drug's anticonvulsant effects.

Since Killam et al's (1973) seminal report, there have been many reports of tolerance to DZP's anticonvulsant effect on a variety of different types of convulsions. For example, Baltzer et al. (1980) reported tolerance to the anticonvulsant effects of DZP (5 mg/kg to 15 mg/kg, PO) on maximal electroshock convulsions in rats after just 5 days of treatment; these authors noted that tolerance was not expressed in a consistent fashion, but instead waxed and waned on a 10-day cycle. Interestingly, a similar pattern is apparent in the data reported by Killam et al. (1973), and this phenomenon is described in detail by Koella (1986). File (1983) reported the development of tolerance to the anticonvulsant effect of DZP (4 mg/kg, IP) on picrotoxin- or pentylenetetrazol-induced convulsions in mice after as few as 10

days. Löscher and Schwark (1985) reported the development of tolerance to DZP's anticonvulsant effect (5 mg/kg, IP, 3 times per day for 14 days) on kindled convulsions in rats. Concomitant determination of plasma concentrations of DZP revealed that the levels of DZP actually increased over the 2-week treatment period, suggesting that a functional rather than dispositional change was responsible for the development of tolerance. Finally, Schneider and Stephens (1988) reported that tolerance develops to DZP's (5 mg/kg or 20 mg/kg, IP, for 9 days) anticonvulsant effects on convulsions elicited by the benzodiazepine inverse agonist FG 7142, a drug that also binds at the benzodiazepine receptor but elicits effects that are opposite (e.g., proconvulsant; anxiogenic) to those elicited by benzodiazepine agonists such as DZP.

Tolerance to the Anticonvulsant Effects of Valproic Acid

History Although valproic acid was synthesized in the 1880s, its anticonvulsant effects did not become apparent until Meunier's group (1963; cited in Kupferberg, 1980) made the serendipitous observation that many compounds demonstrated a pronounced anticonvulsant effect upon PTZ convulsions when they were administered in a valproic acid vehicle. Meunier's conclusion that the anticonvulsant effect was due to the vehicle, rather than to the drugs themselves, has since been confirmed many times and today sodium valproate (VPA), the sodium salt of valproic acid, is widely used in the treatment of absence seizures,

partial seizures, and generalized tonic-clonic seizures (see Löscher, 1985; Engel, 1989).

Pharmacologic Profile VPA is a hygroscopic white powder that dissolves easily in water or ethanol but less readily in solvents such as acetone. It is rapidly absorbed following IP or PO administration, with peak plasma levels being reached within 30 min of administration (e.g., Morton, 1984). VPA enters the CNS via an active transport mechanism; consequently, brain levels of VPA may lag behind plasma concentrations (see Nau & Löscher, 1978). Once it enters the brain, VPA is homogeneously distributed and there is little evidence of drug accumulation following repeated administration (see Löscher, 1985). VPA has a half-life of about 4.5 hr in the rat; hepatic metabolism is the major route of elimination. Löscher (1981) has demonstrated that many of VPA's metabolites have anticonvulsant activity of their own, though only 2-en valproic acid occurs in significant levels in the brain under therapeutic conditions (see Löscher, 1985).

VPA is noted for the pronounced and often unpredictable interactions that it can have on the effects of other drugs (Henriksen & Johannessen, 1984). For example, the dose of phenobarbital can be cut in half with no loss of plasma concentration of the drug when VPA is added to therapy because VPA interferes with the breakdown of phenobarbital. Conversely, it is often difficult to establish therapeutic plasma concentrations of VPA when it is coadministered with

antiepileptic drugs such as phenytoin or CBZ because these drugs facilitate the breakdown of VPA (Eadie, 1985; Henriksen & Johannessen, 1984). For this reason, monotherapy may be preferable when VPA is used in the treatment of epilepsy (Henriksen & Johannessen, 1984).

The mechanism of VPA's anticonvulsant action is unknown. Löscher (1985) suggested that VPA's anticonvulsant effect is attributable to an increase in synaptosomal levels of the inhibitory neurotransmitter GABA, which would subsequently increase the amount available for release from these neurons. More recently, Löscher (1989) reported that levels of GABA in GABAergic neurons with terminal fields in the substantia nigra are particularly enhanced by VPA. Interestingly, GABAergic ennervation of the substantia nigra has been implicated as a critical factor in the ontogeny of seizures (e.g., Gale, 1988; McNamara, Galloway, Rigsbee, & Shin, 1984). It has also been suggested that VPA mediates its anticonvulsant effects by a direct postsynaptic enhancement of GABAergic transmission similar to that demonstrated by the benzodiazepines (see Jurna, 1985). However, Löscher (1989) pointed out that a facilitation of GABAergic transmission is only observed at VPA concentrations that are far greater than those required for anticonvulsant effects (see also McLean & Macdonald, 1986). Alternatively, the anticonvulsant effect of VPA has been attributed to a reduction in the release of the putative neuromodulator gamma-hydroxybutyrate (e.g., Vayer, Cash, & Maitre, 1988), a product of

GABA breakdown that has been shown to elicit seizures in both cats and rats (see Vayer et al., 1988). Finally, VPA's anticonvulsant effect may also be related to its voltage- and use-dependent inhibitory effect on sodium-dependent, high-frequency repetitive firing of cultured cortical neurons (McLean & Macdonald, 1986). Although the basis for this inhibitory effect is unknown, McLean and Macdonald (1986) suggested that it may be related to VPA's ability to decrease sodium conductances. Alternatively, Morre, Keane, Vernières, Simiand, and Ronucci (1984) suggested that VPA's anticonvulsant effect is due to its ability to increase potassium currents, although this effect is only apparent at VPA concentrations that are at least an order of magnitude greater than those required to produce an anticonvulsant effect (Johnston, 1984).

Anticonvulsant Activity VPA displays a broad spectrum of moderate activity against a number of different types of convulsions (see Löscher, 1985). For example, Tulloch, Walter, Howe, and Howe (1982) reported that VPA (200 mg/kg to 800 mg/kg, IP) had an anticonvulsant effect on maximal electroshock, pentylenetetrazol-induced, and kindled convulsions in rats. VPA has also been shown to attenuate audiogenic convulsions in rats (Consroe et al., 1980) and mice (e.g., Anlezark, Horton, Meldrum, & Sawaya, 1976). Meldrum, Anlezark, Ashton, Horton, and Sawaya (1977) found that VPA attenuated photically induced convulsions in the baboon Papio papio at doses of 100 mg/kg to 200 mg/kg, IV. And VPA (150 mg/kg to 400 mg/kg, SC) has been shown to reduce the

convulsions elicited by bicuculline, picrotoxin, and strychnine (see Kupferberg, 1980).

Clinically, VPA is the drug of choice for the treatment of mixed seizure disorders. It is most effective against generalized convulsions but also used to control partial seizures (Engel, 1989). VPA is especially useful when sedative side-effects are undesirable becaue it produces little sedation at therapeutic doses (Engel, 1989); however, the remote possibility of hepatotoxic side effects limits its widespread acceptance as a drug of first choice in the treatment of the epilepsies (see Eadie, 1985; Engel, 1989).

Tolerance to the Anticonvulsant Effects of VPA Although clinical reports of tolerance to VPA's anticonvulsant effect have existed since the early 1970's (see Frey, 1985) the phenomenon has been difficult to demonstrate in the laboratory. Lockard, Levy, Congdon, DuCharme, and Patel (1977) found that a low dose of VPA (administered IV at a rate that maintained plasma concentrations at between 50 micrograms/ml to 150 micrograms/ml for up to 6 weeks) frequently failed to provide a sustained anticonvulsant effect on alumina-gel-induced seizures in the baboon; however, it is not clear whether this loss of efficacy was due to fluctuations in plasma levels of the drug or to the development of genuine tolerance. Paule and Killam (1986) subsequently reported the development of tolerance to VPA's anticonvulsant effect (increasing doses from 7.5 mg/kg to 240 mg/kg per day, PO, spread over three equal doses, for up to 12 weeks) on photic

seizures in P. papio; they found that the expression of tolerance to VPA's anticonvulsant effect could not be prevented by increasing the treatment dose.

There have been several clinical reports of tolerance to VPA's anticonvulsant effect. For example, Bruni and Albright (1983) reported that tolerance readily to VPA's anticonvulsant effect on complex partial seizures; they found that the development of tolerance proceeded most readily in patients with the highest frequency of seizures prior to the initiation of treatment. Similarly, Meinardi, Smits, and van den Brink (1986) reported a rapid loss in VPA's efficacy in patients with intractable epilepsy; however, these authors questioned whether the loss of efficacy was actually due to the development of tolerance or to an exacerbation of the patients' condition.

These positive demonstrations of tolerance to VPA's anticonvulsant effects are tempered by a number of reported failures to observe such tolerance. For example, Young, Lewis, Harris, Jarrott, and Vadja (1987) found no evidence of tolerance to VPA's anticonvulsant effects (200 mg/kg, IP, twice a day) on kindled convulsions in rats after 12 treatment days. Similarly, Gent, Bently, Feely, and Haigh (1986) failed to demonstrate the development of tolerance to VPA's anticonvulsant effect on pentylenetetrazol-induced convulsions, and Löscher (1986b) found no evidence of tolerance to VPA's anticonvulsant effects (600 mg/kg/day, in drinking water, or continuous administration with osmotic minipumps) on electroshock convulsions in mice or

audiogenic convulsions in gerbils.

Summary: Tolerance to Anticonvulsant Drug Effects

The development of tolerance to the anticonvulsant effects of antiepileptic drugs such as CBZ, DZP, and VPA has been clearly and repeatedly demonstrated. However, this work has been guided by the conventional premise that drug tolerance is a pharmacologic phenomenon, sensitive only to the manipulation of variables associated with drug administration: for example, the size of the treatment dose (e.g., Killam et al., 1973), the interdrug interval (e.g., Frey, 1987), and the length of the treatment period (e.g., Baltzer et al., 1980). The possibility that convulsive activity during periods of drug exposure could play an important role in the development of tolerance to the anticonvulsant effects of antiepileptic drugs has not been entertained by the researchers in this area. As described in the next two sections of this Introduction, this oversight provided the initial motivation for the present thesis.

6. General Rationale

There were three general reasons for my initial interest in the characterization of contingent and pharmacologic tolerance to the anticonvulsant effects of antiepileptic drugs. First, such integrative work is necessary to further our understanding of these two phenomenologically distinct forms of tolerance. As Goudie and Emmett-Oglesby (1989) noted:

> "It is important to know how the relative significance of various mechanisms that produce tolerance...may be modified by basic pharmacological variables, on the one hand (such as the drug studied, drug dose, frequency of dosing, duration of dosing, route of dosing, and so on) and on the other hand, by behavioral variables (such as the specific behaviors studied and the environmental and behavioral contexts within which they are studied)." (p. 5).

The present thesis was motivated by a similar desire for a better understanding of the relation between contingent and pharmacologic drug tolerance.

The second reason for my interest in the phenomena of contingent and pharmacologic tolerance to the anticonvulsant effects of antiepileptic drugs stems from the clinical implications of such knowledge. For example, Haigh and Feely (1988) have pointed out that the wide therapeutic window, broad spectrum of activity, and remarkable lack of side-effects combine to make the benzodiazepines an ideal pharmacologic treatment for the epilepsies; however, these advantages are outweighed by the fact that tolerance rapidly develops to the anticonvulsant effects of these drugs. Accordingly, a better understanding of the factors involved in the development of tolerance to antiepileptic drugs might assist the development of new antiepileptic compounds that are less prone to the development of tolerance, as well as the more informed prescription of existing

drugs so that the problems associated with the development of tolerance are minimized (see also Baltzer et al., 1980; Koella & Meinardi, 1986).

The third reason for my interest in the relation between contingent and pharmacologic tolerance to the anticonvulsant effects of antiepileptic drugs arose from the fact that the kindling paradigm that we have developed to study tolerance to anticonvulsant drug effects is well suited to studying the relation between contingent and pharmacologic tolerance--in fact, my colleagues and I had already used the paradigm to demonstrate both contingent and pharmacologic tolerance to the anticonvulsant effects of ethanol (Mana, Pinel, & Lê, 1988; Mana, Lê, Kalant, & Pinel, in preparation). The next section describes the kindling phenomenon in general as well as the general methodology for all five experiments in the present thesis.

II. GENERAL METHODS

The Kindling Paradigm

Periodic electrical stimulation of the rat amygdala at intensities capable of eliciting afterdischarges but no overt convulsions leads to a progressive intensification of the afterdischarges and to their convulsive effects; after approximately 15 such stimulations each rat will respond to each stimulation with a fully generalized electrographic and motor seizure (see Goddard, 1967; Goddard, McIntyre & Leech, 1969; Racine, 1972a; 1972b; McNamara, 1988). This phenomenon is referred to as kindling (Goddard et al., 1969); it has subsequently been shown that kindling can be elicited by both chemical and electrical stimulation of a wide variety of brain sites in many different species (for reviews, see McNamara, 1988; Racine & Burnham, 1984) although the majority of kindling experiments have involved electrical stimulation of the amygdala in rats (Racine & Burnham, 1984).

The kindling model has emerged as a useful tool for assessing the anticonvulsant effects of a wide variety of drugs (e.g., Albertson, Peterson, & Stark, 1980; Albright & Burnham, 1980). It has been shown that clinically prescribed antiepileptic drugs may attenuate the intensity and duration of kindled convulsions and of the underlying afterdischarges. It is important to recognize that the various electrographic and behavioral indices of the kindled seizure are not all affected in the same way by every anticonvulsant drug; Racine and Burnham

(1984) have argued that the varying sensitivity of different aspects of the kindled seizure to anticonvulsants may model the sensitivity of different types of human epilepsy to these same drugs.

Given the utility of the kindling paradigm in the study of the anticonvulsant effects of a wide variety of drugs (e.g., Albright & Burnham, 1980; Ashton & Wauquier, 1979; Pinel, 1983; Schmidt, 1987), it is surprising that it has only recently been adopted to study the development of tolerance to anticonvulsant drug effects. Kindled convulsions have three important advantages over the experimental convulsions that have traditionally been employed to assess the development of tolerance to anticonvulsant drugs--specifically, over convulsions induced by maximal electroshock stimulation (MES) or pentylenetetrazol (PTZ). Both MES and PTZ induce convulsions that are variable in form and duration, are difficult to measure, and in the case of PTZ, often associated with subject injury or fatality (e.g., Swinyard, 1980; Voskuyl et al, 1986). This latter problem is particularly serious in studies of tolerance in which anticonvulsant effects are repeatedly assessed in the same subjects, because any systematic change in the apparent anticonvulsant action of a drug is always confounded by the progressive debilitation and attrition of those subjects experiencing the most severe convulsions. In contrast, kindled rats remain healthy and easy to handle for the duration of an experiment, and fatalities are rare. Moreover, in well-kindled

rats it is possible to elicit convulsions that vary little from subject to subject in either form or duration, and baselines can be established in individual animals that display almost no fluctuation from stimulation to stimulation (see Pinel, Phillips, & MacNeil, 1973). The importance of such reliability in the study of tolerance to the effects of anticonvulsant drugs is obvious, and the stereotyped nature of kindled motor convulsions makes it easy to measure their intensity.

The following sections describe the kindling paradigm that we have developed to study anticonvulsant drug effects and other methodology common to each of the experiments in the present thesis. Any variations to this general methodology are described in the methods sections of the respective experiments.

Subjects. The subjects in all of the experiments were male hooded rats (Charles River, Canada), weighing 350 g to 450 g at the time of surgery. The rats were individually housed in wire mesh cages with continuous access to food and water. Each experiment was conducted at approximately the same time (+/- 2 hr) during the light phase of the 12/12 hr light/dark cycle.

Surgical Procedure. A single bipolar electrode (Plastic Products MS-303-2) was implanted in the left basolateral amygdaloid nucleus of each rat, 2.8 mm posterior to bregma, 5 mm lateral and 8.5 mm ventral to the skull surface at bregma, with the incisor bar set at -3.3 (coordinates taken from Paxinos & Watson, 1982). Tetracycline was sprinkled on the incision before suturing, and it was added to the drinking water for 7 days after surgery.

Kindling. The kindling phase of each study began at least 7 days after surgery. During the kindling phase, each rat was stimulated (1 sec, 60 Hz, 400 microamps) three times per day, 5 days per week, for 3 weeks. There were at least 2 hr between consecutive stimulations. Prior to each stimulation, the stimulation lead was connected and the subject was placed in a 58 X 58 X 25 cm opaque plastic chamber containing a 5-cm layer of San-i-cel bedding material. The stimulation was delivered immediately, and the rat was returned to its home cage once the behavioral signs of convulsive activity ceased. As is usual, the only behavioral effect of the initial stimulations was a brief period of behavioral arrest, but by the end of the kindling phase each stimulation elicited a generalized clonic convulsion characterized in sequence by facial clonus, forelimb clonus, repeated bouts of rearing and a loss of equilibrium (see Pinel & Rovner, 1978; Racine, 1972b).

Stimulation-Baseline Phase. In each study, the stimulation-baseline phase began 48 hr after the completion of the kindling phase. During the stimulation-baseline phase, each rat received four amygdaloid stimulations, one every 48 hr. The duration of forelimb clonus elicited by each stimulation was recorded as the dependent measure.

Drug-Baseline Trial. The drug-baseline trial occurred 48 hr after the fourth and final stimulation-baseline trial. On the drug-baseline trial of each experiment, each subject received an IP injection of one of the drugs (either CBZ, DZP, or VPA) 1 hr

prior to the delivery of the convulsive stimulation. The duration of forelimb clonus elicited by the stimulation was recorded for each subject. This was done to determine each subject's initial sensitivity to the drug's anticonvulsant effect. Subjects not displaying at least an 80% reduction in the duration of forelimb clonus from the fourth stimulation-baseline trial to the drug-baseline trial were dropped from the study at this point. After the drug-baseline trial, the remaining rats in each experiment were assigned to groups in such a way that the mean durations of forelimb clonus for each group on both the fourth stimulation-baseline trial and the drug-baseline trial were approximately equal.

Tolerance-Development Trials. Because the tolerance-development phase varied from experiment to experiment, a description of the tolerance-development phase is included in the methodology section of each experiment.

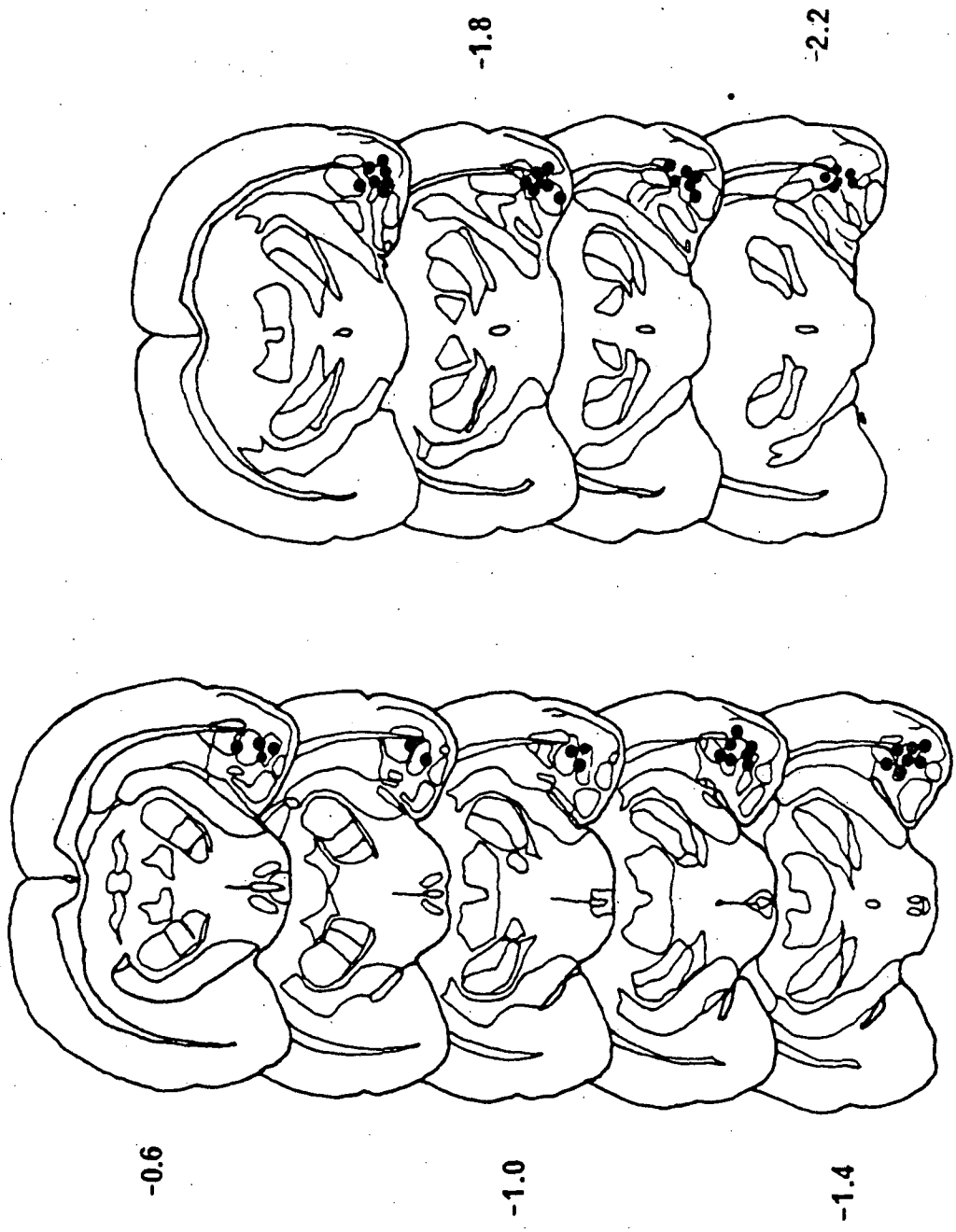
Tolerance-Test Trial. The methodology for the tolerance-test trial was identical to that described for the drug-baseline phase; each subject received an IP injection of the appropriate drug (either CBZ, DZP, or VPA) 1 hr prior to the delivery of the convulsive stimulation. The duration of forelimb clonus elicited by the test stimulation was recorded for each subject.

Histology. At the end of each experiment, all the subjects were sacrificed in a CO2 chamber according to Canada Council on Animal Care guidelines and their brains were removed. A representative sample (20%) of the brains from each experiment were sectioned at

60 microns and stained with luxol blue to permit histological verification of electrode sites (see Figure 3). All electrode sites were located within the amygdaloid complex, with the majority lying within the basolateral amygdaloid nucleus (see Figure 3).

Statistical Analyses. Unless otherwise noted, the data from the drug-baseline trial and the tolerance-test trial for each experiment were analyzed in a between-within, repeated-measures analysis of variance (ANOVA). Significant interactions were further assessed with tests of simple main effects (Kirk, 1968), so that the contributions of the respective between- and within-group factors to the interaction could be determined. When there were more than two factors involved in a signficant test of main effects, Neuman-Keuls posthoc comparisons were performed to determine the contribution of each factor to the significance. Alpha was maintained at the .05 level for the repeated-measures ANOVAs and the tests of simple main effects, as well as the Neuman-Keuls post hoc comparisons.

Figure 3. Representative electrode placements for the rats in each of the five experiments from the present thesis. Although histological verification was completed on a total of 65 subjects (20% of the rats that completed one of the five experiments), the placements for only 50 rats are presented so that their relative placements can be established. The remaining placements were within the boundaries of the placements that are presented, in the sections between -1.0 mm and -2.0 mm from bregma.


-1.8
-2.2
-0.6
-1.0
-1.4

III. Experiment 1

In Experiment 1, we used the kindling paradigm developed by Pinel and his colleagues to study the development of tolerance to the anticonvulsant effects of three widely prescribed antiepileptics: CBZ, DZP, and VPA. These drugs were chosen for four reasons. First, each of the three drugs is effective against generalized tonic-clonic epileptic seizures in humans (see Schmutz, 1985; Löscher, 1985; Haigh & Feeley, 1988), which are closely modelled by generalized kindled convulsions in rats (see Racine & Burnham, 1984). Second, each has been shown to exert a reliable anticonvulsant effect on kindled convulsions (e.g., Albertson, Peterson & Stark, 1980; Albright & Burnham, 1980). Third, each drug is representative of a different family of antiepileptic with different putative mechanisms of action. And fourth, there is a marked difference in the prevalence of previous reports that tolerance will develop to the anticonvulsant effects of these drugs: There have been many experimental and clinical reports of tolerance to DZP's anticonvulsant effect, relatively fewer reports of tolerance to CBZ's anticonvulsant effect, and so few reports of tolerance to VPA's anticonvulsant effect that its existence has recently been questioned (Haigh & Feely, 1988).

Previous attempts to demonstrate the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions have produced equivocal results. For

example, Löscher and Schwark (1985) reported only a small attenuation in the anticonvulsant effect of DZP (5 mg/kg, IP, administered every 8 hr for 10 days); they found that the convulsions elicited on the final test trial were generally limited to bouts of facial clonus and head bobbing (i.e., Class 2 convulsions according to Racine's [1972b] scale). Similarly, Hönack and Löscher's (1988) report of tolerance to CBZ's anticonvulsant effect is tempered by a lack of tolerance to CBZ's effects on the mean afterdischarge duration and by the fact that the drug maintained an inconsistent but obvious anticonvulsant effect on all dependent measures throughout the tolerance-development phase--even on the last trial before the final test trial. And equally equivocal results provided the basis for Young et al.'s (1987) claim that tolerance does not develop to VPA's anticonvulsant effect on kindled convulsions; although there was little evidence of tolerance on the final test trial there was evidence of a substantial decrease in VPA's anticonvulsant effect during the tolerance-development phase.

The equivocal nature of the previous reports of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions leave unanswered the question "Does tolerance develop to the anticonvulsant effects of these drugs on kindled convulsions?". Given the importance of this question to the remaining experiments in the present thesis, the purpose of Experiment 1 was to determine whether tolerance develops to the

anticonvulsant effect of CBZ, DZP, and VPA on kindled convulsions in the rat.

Methods

Subjects. The subjects were 74 male Long Evans rats, weighing between 350 g and 400 g at the time of surgery and between 550 g and 650 g at the completion of the experiment.

Drugs. All drugs were administered intraperitoneally, in a 2% Tween-80/ isosaline vehicle, at a volume of 4 ml/kg. The DZP (2 mg/kg; purchased as Valium, in ampoule form, from Hoffman-LaRoche) was injected in solution; both VPA (250 mg/kg; purchased as Depakote from Abbott Laboratories) and CBZ (75 mg/kg; purchased as Tegretol from Geigy) were injected as suspensions. These doses were selected on the basis of pilot data which indicated that they represented an ED(90) for suppression of forelimb clonus in kindled rats following an acute injection.

Stimulation-Baseline Phase. The stimulation-baseline phase began 48 hr after the completion of the kindling phase. Rats that did not demonstrate at least 20 sec of forelimb clonus on the last stimulation-baseline trial were not studied further (n = 9).

Immediately after the stimulation-baseline phase, the subjects were assigned to one of three different groups--either a CBZ group, a DZP group, or a VPA group--in such a way that the average duration of forelimb clonus for each group on the last stimulation-baseline trial was approximately equal.

Drug-Baseline Trial. On the drug-baseline trial, the rats from

one of the groups received DZP 1 hr before the scheduled convulsive stimulation; those from the second group received VPA; and those from the remaining group received CBZ. Rats not showing at least an 80% decrease in forelimb clonus duration on the drug-baseline trial relative to the last trial of the stimulation-baseline phase were not studied further; two rats receiving VPA, two rats receiving DZP, and one rat receiving CBZ did not meet this criterion for inclusion. Thus, 60 rats remained in the experiment at the start of the tolerance-development phase.

The remaining rats from each of the three drug groups were then assigned to one of two conditions--either a drug condition or a vehicle-control condition--so that the mean forelimb clonus durations on both the last trial of the stimulation-baseline phase and the drug-baseline trial were approximately equal for the resulting six groups.

Tolerance-Development Phase. The tolerance-development trials began 48 hr after the drug-baseline trial. During each of the ten tolerance-development trials, each rat was removed from its home cage, weighed, and the appropriate dose of drug (DZP, n = 12; VPA, n = 12; or CBZ, n = 12) or vehicle (DZP-Control, n = 8; VPA-Control, n = 8; CBZ-Control, n = 8) was administered 1 hr before the scheduled convulsive stimulation.

Tolerance-Test Trial. The tolerance-test trial occurred 48 hr after the last tolerance-development trial and was identical to

the drug-baseline trial; that is, the rats in each drug group and its respective control group received the appropriate drug 1 hr before a convulsive stimulation.

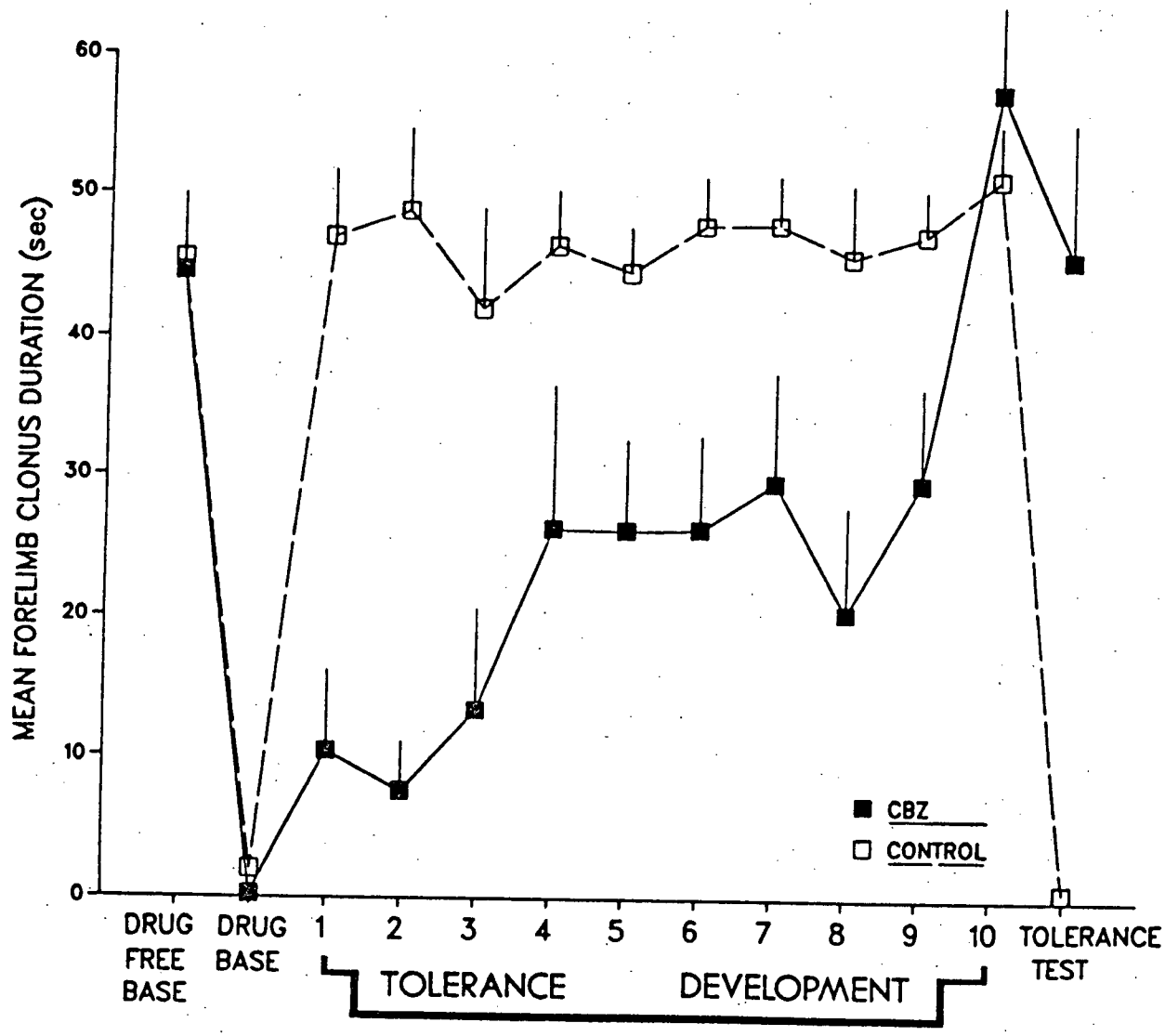
Statistics. Separate 2 (Groups) X 2 (Trials) between-within, repeated-measures analyses of variance were used to analyze the data from the drug-baseline trial and the tolerance-test trial for each of the three drugs. Analyses of simple main effects were used to assess the significance of the different between- and within-groups factors for each significant interaction.

Results

Tolerance developed to the anticonvulsant effects of CBZ, DZP, and VPA in the three drug groups but not in the three vehicle-control groups. As illustrated in Figure 4 (CBZ), Figure 5 (DZP), and Figure 6 (VPA), each of the three drugs almost totally suppressed forelimb clonus in each of the groups on the drug-baseline trial. In contrast, on the tolerance-test trial the drugs had little anticonvulsant effect on the rats from each of the three drug groups, whereas the rats from the three vehicle control groups displayed no tolerance whatsoever.

The statistical analyses confirmed the significance of the effects summarized in Figure 4, Figure 5, and Figure 6. The analyses of variance revealed a significant group X trial interaction for each of the three drugs: CBZ, F (1, 18) = 14.83; DZP, F (1,18) = 21.27; VPA, F (1,18) = 11.48; all p's < .01. Subsequent analysis of each significant interaction using

Figure 4. Tolerance to the anticonvulsant effects of CBZ. On the drug-baseline trial, CBZ exerted a potent anticonvulsant effect on all of the subjects. However, on the tolerance-test trial the rats from the CBZ group displayed substantial tolerance to the anticonvulsant effects of the drug. In contrast, there was no evidence of tolerance in any of the CBZ-Control subjects.


MEAN FORELIMB CLONUS DURATION (sec)
60
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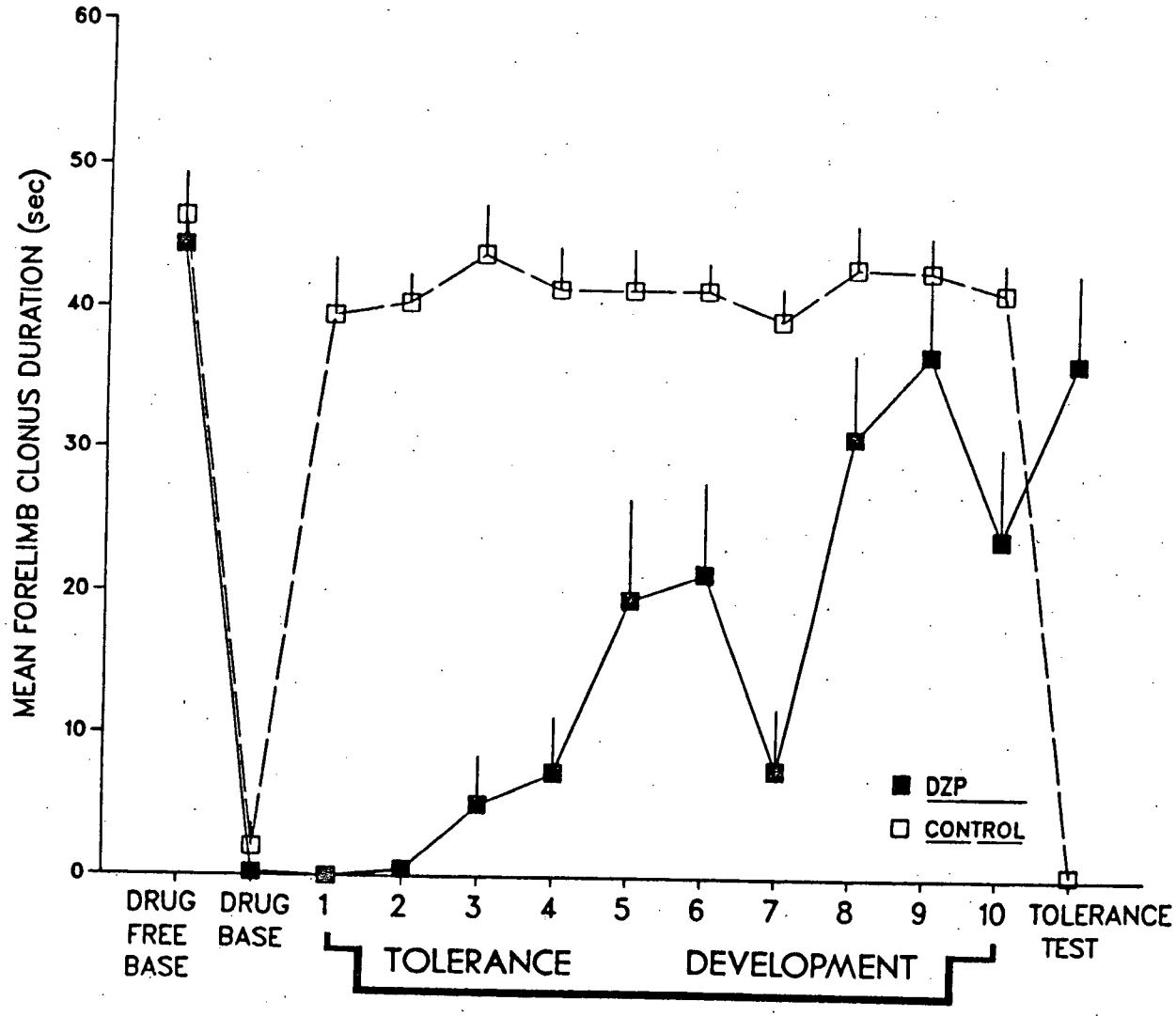
30
20
10
0
CBZ
CONTROL
DRUG FREE BASE
DRUG BASE
1
2
3
4
5
6
7
8
9
10
TOLERANCE TEST
TOLERANCE DEVELOPMENT

Figure 5. Tolerance to the anticonvulsant effects of DZP. On the drug-baseline trial, DZP exerted a potent anticonvulsant effect on all of the subjects. However, on the tolerance-test trial the rats from the DZP group displayed substantial tolerance to the anticonvulsant effects of the drug. In contrast, there was no evidence of tolerance in any of the DZP-Control subjects.


MEAN FORELIMB CLONUS DURATION (sec)
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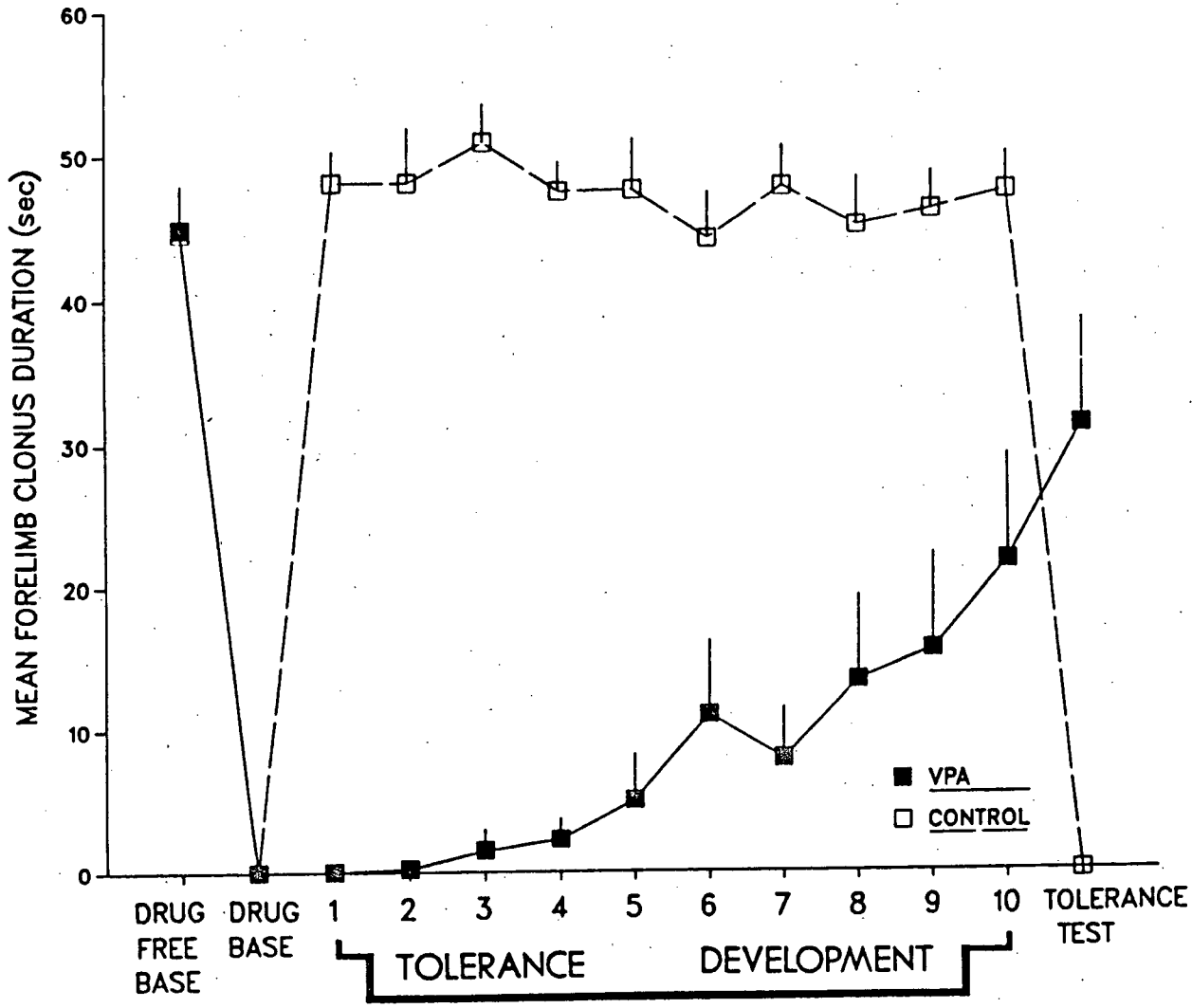
50
40
30
20
10
0
DRUG FREE BASE
DRUG BASE
1
2
3
4
5
6
7
8
9
10
TOLERANCE TEST
TOLERANCE DEVELOPMENT
DZP
CONTROL

Figure 6. Tolerance to the anticonvulsant effects of VPA. On the drug-baseline trial, VPA exerted a potent anticonvulsant effect on all of the subjects. However, on the tolerance-test trial the rats from the VPA group displayed substantial tolerance to the anticonvulsant effects of the drug. In contrast, there was no evidence of tolerance in any of the VPA-Control subjects.


MEAN FORELIMB CLONUS DURATION (sec)
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10
0
VPA
CONTROL
DRUG FREE BASE
DRUG BASE
1
2
3
4
5
6
7
8
9
10
TOLERANCE TEST
TOLERANCE DEVELOPMENT

analyses of simple main effects revealed a significant increase in the duration of forelimb clonus between the drug-baseline trial and the tolerance-test trial for the rats from the CBZ group (F (1,11) = 21.59, p < .001), the DZP group (F (1,11) = 32,=.71, p < .001), and the VPA group (F (1,11) = 17.54, p < .01). In contrast, tests of simple main effects revealed no significant differences in forelimb clonus duration between the drug-baseline trial and the tolerance-test trial for the rats from the three respective control conditions (all p's > .40). Finally, tests of simple main effects indicated that the rats from the three drug groups displayed signficantly more forelimb clonus on the tolerance-test trial than the rats from the three respective control conditions (CBZ: F (1,18) = 18.53; DZP: F (1,18) = 28.19; VPA: F (1,18) = 15.31; all p's < .01).

Discussion

The present demonstrations of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions in the rat are important for two reasons. First, they confirm and strengthen earlier reports of tolerance to the anticonvulsant effects of DZP (Löscher & Schwark, 1985) and CBZ (Hönack & Löscher, 1989) on kindled convulsions in the rat, and they provide conclusive evidence of tolerance to VPA's anticonvulsant effect on kindled convulsions. Second, the present results provide further evidence of the utility of the kindling model as a useful tool in the study of tolerance to the

anticonvulsant effects of antiepileptic drugs.

It is not clear from the present experiment whether a metabolic or a functional change underlies the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA. Hönack and Löscher (1988) suggested that the development of tolerance to CBZ's anticonvulsant effect was attributable to both a dispositional and a functional change; rats that had received CBZ on a chronic basis were able to metabolize the drug significantly faster than drug-naive rats (suggesting a dispositional change), but the drug-experienced rats were also less affected by CBZ than drug-naive rats experiencing equal plasma concentrations of the drug (supporting a functional change). Although the resolution of this question is beyond the scope of this paper, the results of Experiment 2 support the notion that a functional change underlies the development of tolerance to the anticonvulsant effects of all three drugs.

Perhaps the most interesting aspect of the present results is the fact that they stand in sharp contrast to earlier efforts to study the development of tolerance to the anticonvulsant effects of CBZ (Hönack & Löscher, 1988), DZP (Löscher and Schwark, 1985), and VPA (Young et al., 1987) on kindled convulsions. The magnitude of tolerance that Löscher and his colleagues reported to the anticonvulsant effects of CBZ (Hönack & Löscher, 1988) and DZP (Löscher & Schwark, 1985) on kindled convulsions was markedly less than that demonstrated in the

present experiment; tolerance developed to the anticonvulsant effects of these drugs on only half of the dependent measures they recorded and it was not substantial even on these measures. Furthermore, Young et al. (1987) found no evidence of tolerance to the anticonvulsant effects of VPA (200 mg/kg, IP, administered every 12 hr for 14 days) on kindled convulsions in rats.

There are at least three plausible explanations for the differences between the results of the present experiment and those reported by Löscher and Schwark (1985), Young et al. (1987), and Hönack and Löscher (1988). The first reason concerns obvious differences in the drug-treatment regimens employed in the respective experiments. The doses of CBZ, DZP, or VPA administered in the present experiments were considerably smaller, and/or administered less frequently, than those administered by Löscher and Schwark (1985; they administered DZP 5 mg/kg, IP, every 8 hr, in comparison to 2 mg/kg, IP, every 48 hr, in the present study), Young et al. (1987; they administered VPA 200 mg/kg, IP, every 12 hr, in comparison to 250 mg/kg, IP, every 48 hr in the present study), or Hönack and Löscher (1988; they administered CBZ at a dose of 30 mg/kg, IP, every 8 hr, in comparison to 75 mg/kg, IP, every 48 hr, in the present study). Although the treatment strategy employed by Löscher and Schwark (1985), Young et al. (1987), and Hönack and Löscher (1988) makes sense from the traditional perspective that tolerance is more likely to develop when a high treatment dose is used, it is

accompanied by a liability that is often neglected and may account for the relatively small amount of tolerance reported in their respective papers; the use of a high treatment dose may facilitate the development of tolerance while obscuring its detection, because an accumulation of the drug produces higher plasma levels, and thus a greater drug effect, than would be produced by an acute injection of the same dose (see Kalant et al., 1971).

A second possible explanation for the discrepancy between the present results and those reported by Löscher and Schwark (1985), Young et al. (1987), and Hönack and Löscher (1988), concerns the differences in the kindling protocols used in the various studies. In the present experiment, every subject had demonstrated at least 30 class 5 convulsions (according to Pinel and Rovner's (1978) modification of Racine's (1972b) rating scale) before the tolerance-development phase began. In contrast, Löscher and Schwark (1985) and Hönack and Löscher (1988) began their experiments after their subjects had demonstrated just 10 class 5 kindled convulsions; and Young et al. (1987) consider 2 consecutive stage 5 convulsions to represent a fully kindled state (although it is not clear how many class 5 convulsions the rats in their study had demonstrated before their experiments began). Thus, it is possible that the physiological changes underlying the kindling process were more firmly established in the rats from our experiments than in the

kindled rats used by Löscher and Schwark (1985), Hönack and Löscher (1988), or Young et al. (1987) because they administered fewer convulsion-eliciting stimulations before drug treatment began. As a result, the convulsions in the kindled rats employed in the present experiments may have been more resistant to the anticonvulsant effects of DZP or VPA and therefore less likely to be effectively controlled by these drugs.

A third possible reason for the differences between the present experiment and those reported by Löscher and Schwark (1985), Hönack and Löscher (1988), and Young et al. (1987) is based upon our earlier observation that the development of tolerance to ethanol's anticonvulsant effect on kindled convulsions is often facilitated by the administration of convulsive stimulation during periods of drug exposure (e.g., Pinel et al., 1983; Pinel & Mana, 1986). In the present experiment, each drug injection was followed 1 hr later by a convulsive stimulation; in contrast, this condition was present in only half of the treatment trials in the work reported by Löscher and Schwark (1985) and Young et al. (1987). Thus, the differences between the present results and those reported by Löscher and Schwark (1985) and Young et al. (1987) may reflect the fact that the relation between drug exposure and convulsive stimulation plays an important role in the development of tolerance to the anticonvulsant effects of clinically relevant antiepileptic drugs. This hypothesis was tested in Experiment 2.

IV. Experiment 2

The purpose of Experiment 2 was to determine whether convulsive stimulation during periods of drug exposure would facilitate the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA. As noted earlier, we (e.g., Pinel et al., 1983; 1985; 1989) have demonstrated that convulsive stimulation during periods of drug exposure plays an important role in the development of tolerance to ethanol's anticonvulsant effect. However, there have been no reported attempts to determine whether convulsions have a similar effect on the development of tolerance to the anticonvulsant effects of antiepileptic drugs. Accordingly, the purpose of Experiment 2 was to determine whether the administration of convulsive stimulation during periods of drug exposure can influence the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA.

Methods

Subjects. The subjects were 117 male Long Evans rats, weighing 350 g to 400 g at the time of surgery and 550 g to 650 g at the time of the tolerance-development phase.

Stimulation-Baseline Trial. A total of 8 rats were removed from the experiment because they did not display at least 20 sec of forelimb clonus on the stimulation-baseline trial. Following the stimulation-baseline trial, the subjects were assigned to one of three drug groups--a CBZ group, a DZP group, or a VPA group--so

that the average durations of forelimb clonus for of the three groups on the stimulation-baseline trial were approximately equal.

Drugs. The DZP and CBZ were administered IP, in a 2% Tween-80/isosaline vehicle, at a volume of 4 ml/kg. The DZP (2 mg/kg) was injected in solution, whereas CBZ (70 mg/kg) was injected as a suspension. In contrast to Experiment 1, VPA (250 mg/kg) was administered by gavage in a 2% Tween 80/isosaline vehicle, at a volume of 4 ml/kg. This change in protocol was made because gavage administration caused less distress in the VPA subjects than did the IP injections used in Experiment 1.

Drug-Baseline Trial. On the drug-baseline trial, one group of rats received CBZ; one group received DZP; and the final group received VPA. The appropriate drug was administered to each rat 1 hr before the convulsive stimulation was delivered, and the duration of forelimb clonus recorded. Rats not showing at least an 80% decrease in the duration of forelimb clonus duration displayed on the drug-baseline trial as compared to the last trial of the stimulation-baseline phase were rejected from the study (10 rats receiving VPA; 4 rats receiving DZP; and 4 rats receiving CBZ). Thus, 91 rats remained in the three drug groups at the start of the tolerance-development phase.

The remaining rats from each drug group were then assigned to one of three conditions--either a drug-before-stimulation condition, a drug-after-stimulation condition, or a vehicle-

control condition--so that the mean forelimb clonus durations on both the last trial of the stimulation-baseline phase and the drug-baseline trial were approximately equal for the resulting nine groups.

Tolerance-Development Trials. The tolerance-development trials began 48 hr after the drug-baseline trial. There were a total of ten bidaily (one every 48 hr) tolerance-development trials in each experiment. On each tolerance-development trial, the rats from the drug-before-stimulation condition continued to received CBZ (CBZ-Before-Stimulation, n = 11), DZP (DZP-Before-Stimulation, n = 11), or VPA (VPA-Before-Stimulation, n = 10) 1 hr prior to each stimulation. The rats from the drug-after-stimulation condition (CBZ-After-Stimulation, n = 10; DZP-After-Stimulation, n = 12; or VPA-After-Stimulation, n = 10), received the same dose of the appropriate drug 1 hr after each stimulation. And the rats from the vehicle control condition (CBZ-Control, n = 10; DZP-Control, n = 8; or VPA-Control, n = 9) received a vehicle injection 1 hr before or 1 hr after each stimulation; because the vehicle injections had no effect on the duration of forelimb clonus regardless of whether they were administered before or after the convulsive stimulation, these groups were combined to create a single control group for each drug.

Tolerance-Test Trial. The tolerance-test trial occurred 48 hr after the last tolerance-development trial and followed the same

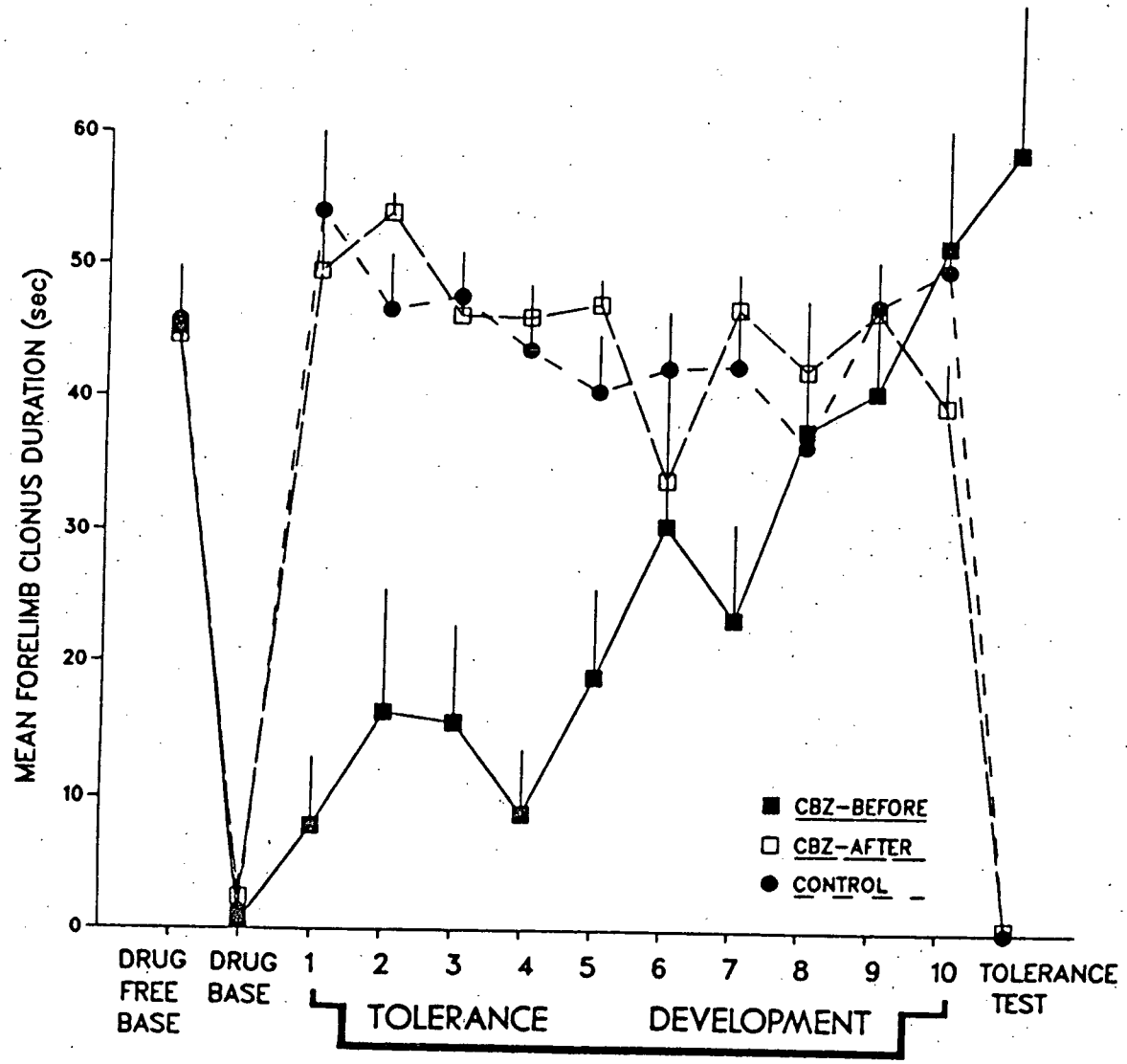
protocol described for the drug-baseline trial; that is, each rat received the appropriate drug 1 hr before convulsive stimulation and the duration of forelimb clonus was recorded.

Statistics. Separate 3 (Groups) X 2 (Trials) between-within factor, repeated-measures analysis of variance were used to analyze the data from the drug-baseline trial and the tolerance-test trial for each of the three drugs. Tests of simple main effects were used to assess the contribution of the respective between- and within-group factors to each signficant interaction.

Results

The results clearly demonstrate that convulsive stimulation can play a key role in the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on amygdaloid-kindled convulsions in the rat. The test doses of CBZ (Figure 7), DZP (Figure 8), and VPA (Figure 9) all exerted a powerful anticonvulsant effect on the kindled rats on the drug-baseline trial; the mean duration of forelimb clonus on this trial was almost zero for each of the nine groups of rats. Over the course of the 10 tolerance-development trials, the rats in each of the drug-before-stimulation groups gradually developed tolerance to the respective drugs' anticonvulsant effects. In contrast, there was little evidence of tolerance displayed by the rats in any of the three drug-after-stimulation groups or the three control groups on the tolerance-test trial; each of the drugs retained its ability to block the forelimb clonus of the rats in these

Figure 7. Contingent tolerance to the anticonvulsant effects of CBZ on amygdaloid kindled convulsions in the rat. On the drug-baseline trial, CBZ exerted a potent anticonvulsant effect on all of the rats. On the tolerance test trial, the rats from the CBZ-Before-Stimulation group displayed substantial tolerance to the anticonvulsant effects of the drug. In contrast, there was no evidence of tolerance demonstrated by the rats from the CBZ-After-Stimulation or the CBZ-Control groups even though the rats in the former group had received the same amount of drug exposure during the tolerance-development phase as the rats from the CBZ-Before-Stimulation group.


MEAN FORELIMB CLONUS DURATION (sec)
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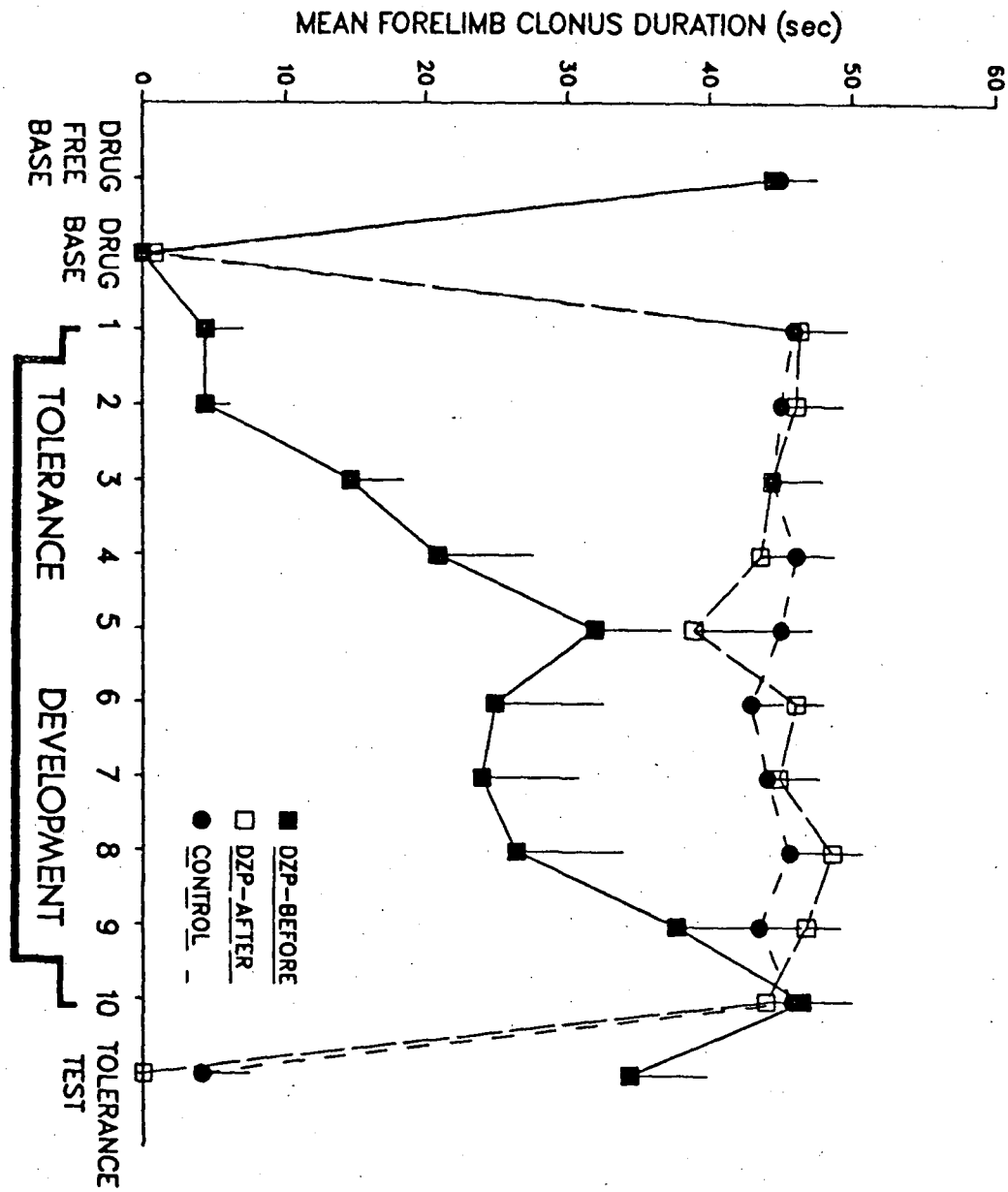
TOLERANCE DEVELOPMENT
TOLERANCE TEST

Figure 8. Contingent tolerance to the anticonvulsant effects of DZP on amygdaloid kindled convulsions in the rat. On the drug-baseline trial, DZP exerted a potent anticonvulsant effect on all of the rats. On the tolerance test trial, the rats from the DZP-Before-Stimulation group displayed substantial tolerance to the anticonvulsant effects of the drug. In contrast, there was no evidence of tolerance demonstrated by the rats from the DZP-After-Stimulation or the DZP-Control groups even though the rats in the former group had received the same amount of drug exposure during the tolerance-development phase as the rats from the DZP-Before-Stimulation group.


MEAN FORELIMB CLONUS DURATION (sec)
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DRUG BASE
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8
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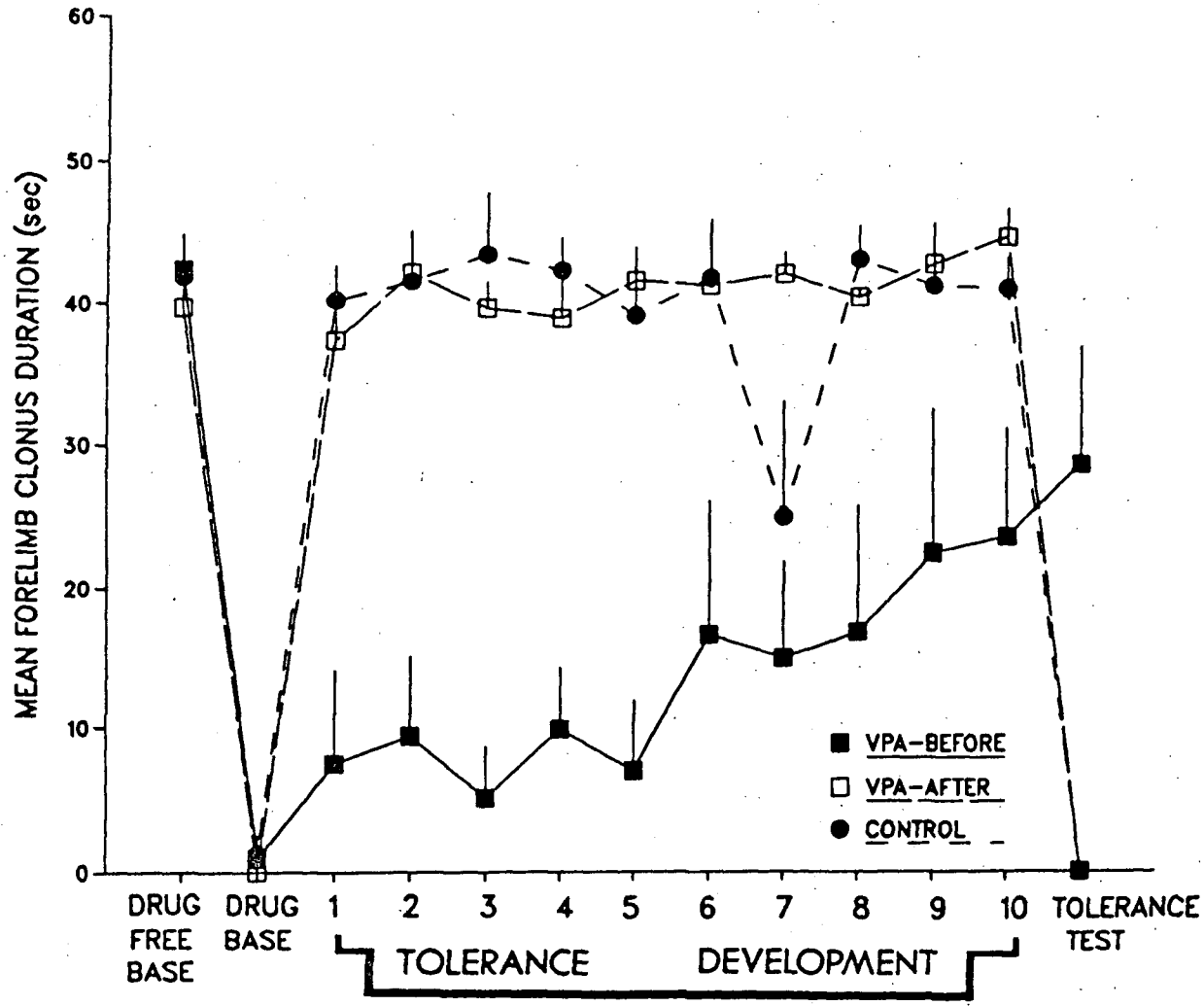
10
TOLERANCE DEVELOPMENT
TOLERANCE TEST
DZP-BEFORE
DZP-AFTER
CONTROL

Figure 9. Contingent tolerance to the anticonvulsant effects of VPA on amygdaloid kindled convulsions in the rat. On the drug-baseline trial, VPA exerted a potent anticonvulsant effect on all of the rats. On the tolerance test trial, the rats from the VPA-Before-Stimulation group displayed substantial tolerance to the anticonvulsant effects of the drug. In contrast, there was no evidence of tolerance demonstrated by the rats from the VPA-After-Stimulation or the VPA-Control groups even though the rats in the former group had received the same amount of drug exposure during the tolerance-development phase as the rats from the VPA-Before-Stimulation group.


MEAN FORELIMB CLONUS DURATION (sec)
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TOLERANCE DEVELOPMENT
TOLERANCE TEST

groups on the tolerance-test trial.

The statistical analyses support the observations made from Figure 7, Figure 8, and Figure 9. The analyses of variance revealed a significant group X trial interaction for each of the three drugs: CBZ, F (2,25) = 30.15; DZP, F (2,27) = 22.82; VPA, F (2,22) = 11.26; all p's < .001. Subsequent tests of simple main effects revealed a significant increase in the duration of forelimb clonus between the drug-baseline and tolerance-test trials for each of the three Drug-Before-Stimulation groups (CBZ-Before: F (1,9) = 34.41; DZP-Before, F (1,10) = 35.64; VPA-Before, F (1,8) = 12.47; all p's < .01). In contrast, tests of simple main effects indicated that there were no signficant differences between the drug-baseline trial and the tolerance test trial for any of the Drug-After-Stimulation or Drug-Control groups (all p's > .5). Accordingly, although tests of simple main effects for the data from the drug-baseline trial revealed no significant differences between each Drug-Before-Stimulation group and the corresponding Drug-After-Stimulation or Drug-Control groups on the final trial of the stimulation-baseline phase or on the drug-baseline trial (all p's > .5), there was a significant difference between the three groups for each drug on the tolerance-test trial (CBZ: F (2,25) = 38.91; DZP: F (2,27) = 29.05; VPA: F (2,22) = 15.75; all p's < .001). Further analysis of the data from the tolerance-test trial for each of the three drugs using Neuman-Keuls posthoc comparisons revealed that the

rats in each Drug-Before-Stimulation group displayed significantly more forelimb clonus than the rats in the corresponding Drug-After-Stimulation or Drug-Control groups (all Neuman-Keuls p's < .05). This pattern of results reflects the development of tolerance to the drugs' anticonvulsant effects in the rats from the CBZ-Before, DZP-Before, and VPA-Before-Stimulation groups.

Discussion

The results of the present experiment are important because they represent the first conclusive evidence that the development of tolerance to the anticonvulsant effects of clinically prescribed antiepileptic drugs can be influenced by the occurrence of convulsive activity during periods of drug exposure. Drug exposure was clearly not a sufficient condition for the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA because there was no evidence of tolerance in the kindled rats from the three drug-after-stimulation groups. In contrast, tolerance developed rapidly to the anticonvulsant effects of each drug when kindled rats were stimulated during each bidaily period of drug exposure.

The fact that the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions in the present experiment was contingent upon convulsive stimulation being delivered during the bidaily periods

of drug exposure, and was not simply a consequence of drug exposure, implies that a functional rather than a dispositional change underlies the development of tolerance in the rats from the respective drug-before-stimulation groups (see also Wolgin, 1989). More importantly, the results of the present experiment provide further support for a drug-effect theory of functional drug tolerance (e.g., Pinel, Kim & Mana, 1990; Pinel & Mana, in press), which emphasizes the idea that functional tolerance is a response to a drug's effect on the activity of the nervous system or other target tissue (see also Jaffe, 1980; Kalant et al., 1971; Kalant, 1985). Consequently, tolerance will develop only to those drug effects that manifest themselves during periods of drug exposure. In many instances, a drug will produce a disruption in the basal activity of the nervous system that is sufficient to initiate the development of tolerance to this effect; that is, the drug effect is an inevitable consequence of drug exposure and tolerance will develop without the recipient engaging in any particular pattern of activity. In other instances, however, the expression and/or magnitude of a particular drug effect is dependent upon the pattern or level of activity of the nervous system of the drug recipient during periods of drug exposure; in these situations, the development of tolerance to the drug effect will be contingent upon the occurrence of a particular pattern or level of neural activity during periods of drug exposure. In the present experiments, the

development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA administered on a bidaily basis was contingent upon the administration of convulsive stimulation during each bidaily period of drug exposure.

The demonstration that the development of tolerance to a given drug effect is contingent upon a particular pattern or level of activity in the nervous system does not imply that tolerance cannot develop to the same drug effect in the absence of such neural activity under a different set of circumstances. For example, the present demonstration that convulsive stimulation plays a key role in the development of tolerance to the anticonvulsant effects of CBZ, DZP and VPA on kindled convulsions does not imply that such stimulation is necessary for the development of all instances of tolerance to the anticonvulsant effects of these or any other drugs. Tolerance to anticonvulsant drug effects has been demonstrated experimentally for nearly every known antiepileptic drug (Frey, 1987)--including CBZ (e.g., Frey & Löscher, 1980), and DZP (e.g., Rosenberg, Chiu, & Teitz, 1986)--in subjects that never experience convulsions until the test trial. These positive results may be due to the chronic drug administration schedule that these studies typically employ; in contrast, the present experiment involved an intermittent, bidaily schedule of drug administration. As mentioned earlier, a schedule of chronic drug administration may facilitate the development of tolerance to a drug's effects (see

Frey, 1987), and in our own laboratory we have used a chronic administration schedule to demonstrate the development of tolerance to the anticonvulsant effects of ethanol in the absence of convulsive stimulation (Mana, Pinel, & Lê, 1988; Mana, Lê, & Pinel, in preparation). Accordingly, a similar schedule of drug administration was used in Experiment 3 to study the development of pharmacologic tolerance to the anticonvulsant effects of DZP on kindled convulsions.

V. GENERAL BACKGROUND FOR EXPERIMENTS 3, 4, AND 5.

The three remaining experiments focus upon the comparison of contingent and pharmacologic tolerance to the anticonvulsant effects of DZP. The decision to restrict the focus of these experiments to a single drug was made for four reasons. The first and most obvious reason was one of economy. The second reason for focusing upon DZP is that tolerance to DZP's anticonvulsant effect is a much more widely recognized experimental phenomenon and clinical problem than is tolerance to the anticonvulsant effects of either CBZ or VPA. Haigh and Feely (1988) noted that the broad spectrum of activity, low toxicity, and virtual absence of peripheral side effects make the benzodiazepines particularly effective anticonvulsants--however, these virtues are negated by the fact that tolerance develops so rapidly to their anticonvulsant effects. Consequently, a considerable amount of attention has been focused upon the phenomenon of tolerance to the anticonvulsant effects of the benzodiazepines in an effort to resolve this problem.

The third reason for studying contingent and pharmacologic tolerance to the anticonvulsant effect of DZP, as opposed to CBZ or VPA, had to do with the fact that more is known about the site and mechanism of action for DZP. The discovery of specific benzodiazepine receptors in the central nervous system (Möhler & Okada, 1977; Squires & Braestrup, 1977) has provided a focus for the study of the mechanisms involved in the development of

tolerance to the effects of the benzodiazepines (see Haigh & Feely, 1988); this advantage does not exist for either CBZ or VPA. Furthermore, the existence of a variety of direct-acting antagonists for the benzodiazepine receptor, which are known to affect the manner in which the benzodiazepines exert their anticonvulsant effect, provides a tool with which to study both pharmacologic and contingent tolerance that is not available with either CBZ or VPA.

The fourth and final reason for restricting the focus of the remaining experiments to the study of contingent and pharmacologic tolerance to DZP's anticonvulsant effect is that more is known about the physiological changes underlying the development of tolerance to its anticonvulsant effect than to the anticonvulsant effect of either CBZ or VPA. The GABA/benzodiazepine/chloride ionophore complex has provided the focus for much of the work in this area. Sher (1983) suggested that a downregulation (decrease in the number) of benzodiazepine receptors following chronic administration of DZP underlies the development of tolerance to its anticonvulsant effect; however, there have been many contradictory reports (e.g., Gallager, Lakoski, Gonsalves, & Rausch, 1984; Möhler, Okada, & Enna, 1978) and the present consensus is that the development of tolerance to the anticonvulsant effects of DZP and other benzodiazepines cannot be fully explained simply in terms of a downregulation of benzodiazepine receptors (e.g., Caccia & Garattini, 1985; Teitz &

Rosenberg, 1988).

Several authors have suggested that a decrease in the sensitivity of the GABA-A receptor following chronic DZP administration can account for the development of tolerance to its anticonvulsant effects. This hypothesis was first proposed by Gallager and her collaborators, based upon their observation that chronic DZP administration decreased the sensitivity of serotonergic neurones in the dorsal raphé nucleus to iontophoretically applied GABA, but not serotonin (Gallager et al., 1984). More importantly, the time course for the emergence and disappearance of GABAergic subsensitivity during chronic administration and withdrawal of DZP was found to roughly correspond to the emergence and dissipation of tolerance to the anticonvulsant effects of the drug (Gonsalves & Gallager, 1987; Teitz & Rosenberg, 1988). The decrease in the functional sensitivity of the GABA receptor following chronic benzodiazepine administration is not accompanied by a decrease in its binding affinity; in fact, although chronic benzodiazepine treatment may decrease the number of GABA receptors in the brain (e.g., Möhler et al., 1978), the affinity of the remaining GABA receptors has been reported to increase (Gallager, Malcolm, Anderson, & Gonsalves, 1985). The decrease in the efficacy of the GABAergic receptor is also associated with a decrease in the GABAergic enhancement of benzodiazepine binding at the benzodiazepine receptor (e.g., Gallager et al., 1984; Teitz, Rosenberg, & Chiu,

1989). This may also contribute to the development of pharmacologic tolerance to DZP's anticonvulsant effect.

VI. Experiment 3

The purpose of Experiment 3 was to replicate Löscher and Schwark's (1985) report of pharmacologic tolerance to DZP's anticonvulsant effect on kindled convulsions in rats. Accordingly, the drug administration regimen used in Experiment 3 was identical to that used by Löscher and Schwark (1985), with one noteable exception. Löscher and Schwark (1985) reported only a moderate amount of tolerance to DZP's anticonvulsant effect; this may be due to the fact that they administered a relatively high treatment dose of DZP (5 mg/kg, IP), every 8 hr for 10 days, and there was a marked accumulation of the drug and its active metabolites over the course of the tolerance-development phase of their experiment. As noted earlier, such an accumulation can obscure the detection of tolerance to a drug's effect because the effective plasma and brain concentration of the drug and its metabolites is much higher in rats receiving chronic drug treatment than would be produced by an acute injection of the same dose of the drug. To reduce the possibility of this occurring in the present experiment, a smaller dose of DZP than that used by Löscher and Schwark (1985) was administered in Experiment 3.

Methods

Subjects. The subjects were 38 male, Long-Evans rats, weighing between 350 g and 400 g at the time of surgery and between 500 g and 600 g at the completion of the experiment.

Drugs. DZP (2 mg/kg, IP) was injected at a volume of 2 ml/kg in a 2% Tween-80/isosaline vehicle.

Stimulation-Baseline Phase. The stimulation-baseline phase began 48 hr after the completion of the kindling phase. A single rat did not demonstrate at least 20 sec of forelimb clonus on the last stimulation-baseline trial and was not studied further.

Drug-Baseline Trial. On the drug-baseline trial, each rat received DZP 1 hr before the scheduled convulsive stimulation. Rats not showing at least an 80% decrease in forelimb clonus duration on the drug-baseline trial relative to the last trial of the stimulation-baseline phase were not studied further (n=7). The remaining 30 rats were assigned to one of two groups--a Pharmacologic-Tolerance group (n = 21) or a Control group (n = 9) --so that the mean forelimb clonus durations for the two groups on both the last stimulation-baseline trial and the drug-baseline trials were approximately equal.

Tolerance-Development Phase. The tolerance-development phase began 24 hr after the drug-baseline trial. During the 10-day tolerance-development phase, the rats in the two groups were not stimulated. The rats in the Pharmacologic-Tolerance group received DZP every 8 hr for 10 days, whereas the rats from the

Control group received vehicle injections on the same schedule.

Tolerance-Test Trial. The tolerance-test trial occurred 8 hr after the last injection of the tolerance-development phase. Each rat received an injection of DZP 1 hr before the delivery of convulsive stimulation. The duration of forelimb clonus elicited by the stimulation was recorded for each rat.

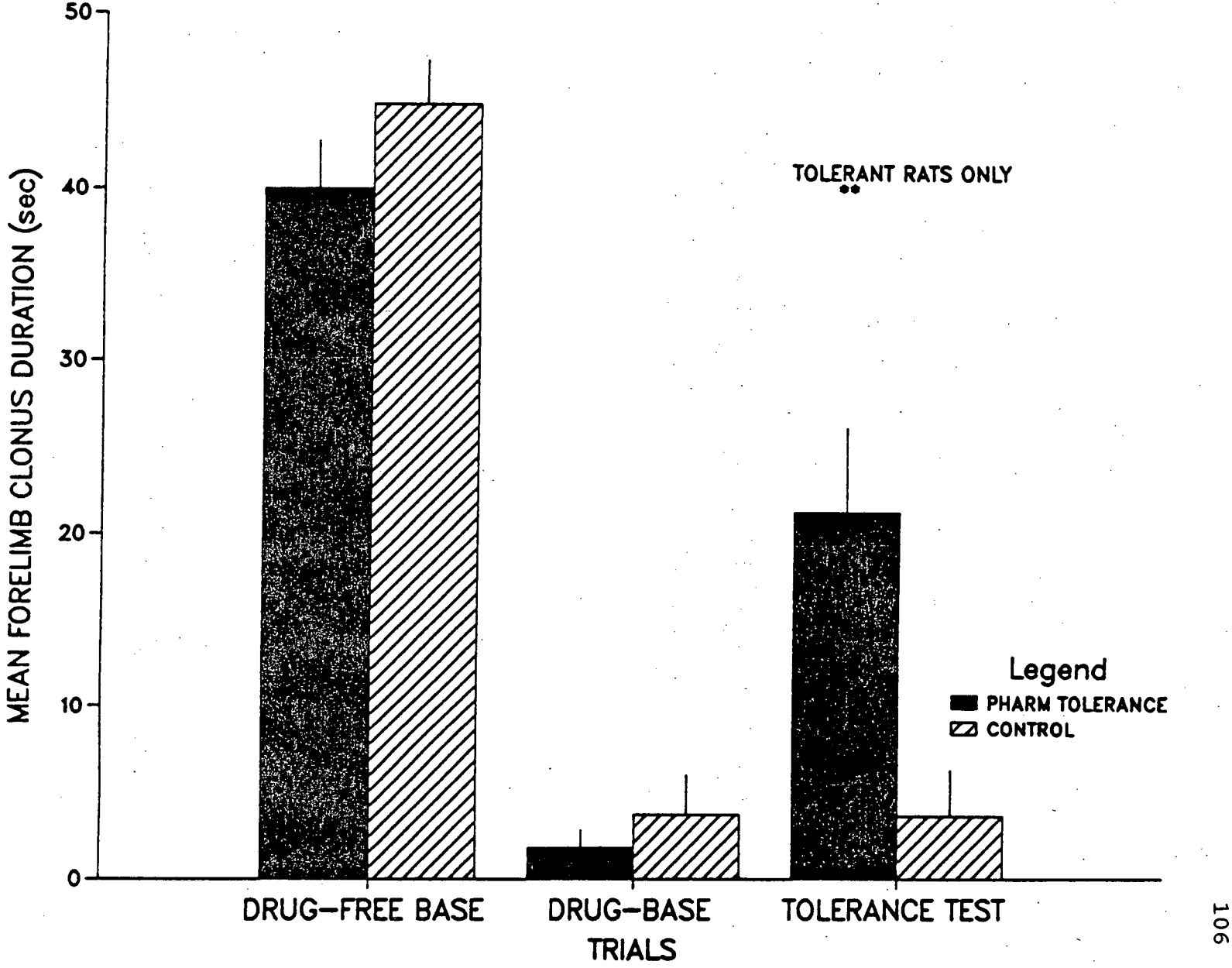
Statistics. A single 2 (Groups) X 2 (Trials) between-within factor, repeated measures analysis of variance was used to analyze the data from the last trial of the drug-baseline trial and the tolerance-test trial. Tests of simple main effects were used to assess the contribution of the respective between- and within-group factors to the significant interaction.

Results

As can be seen in Figure 10, pharmacologic tolerance to DZP's anticonvulsant effect developed in the rats that received DZP every 8 hr during the 10-day tolerance-development phase. The test dose of DZP almost completely suppressed the forelimb clonus of each rat on the drug-baseline trial. On the tolerance-test trial, however, the rats from the Pharmacologic-Tolerance group displayed a small amount of forelimb clonus; in contrast, the test dose of DZP continued to suppress forelimb clonus in almost all of the rats from the Control group.

The statistical analyses support the observations made from Figure 10. The analysis of variance indicated revealed

Figure 10. Pharmacologic tolerance to the anticonvulsant effects of DZP. On the drug-baseline trial, DZP exerted a potent anticonvulsant effect on all of the subjects. On the tolerance-test trial the rats from the Pharmacologic-Tolerance group displayed tolerance to the anticonvulsant effects of the drug. There was no evidence of tolerance in the rats from the Control group on the tolerance-test trial.


50
40
30
20
10
0
MEAN FORELIMB CLONUS DURATION (sec)
TOLERANT RATS ONLY
**
Legend
PHARM TOLERANCE
CONTROL
DRUG–FREE BASE
DRUG–BASE
TOLERANCE TEST
TRIALS

significant group X trial interaction (F (1,28) = 6.16, p = .05). Subsequent analysis of this interaction using tests of simple main effects revealed a significant increase in the duration of forelimb clonus between the drug-baseline and tolerance-test trials for the rats from the Pharmacologic-Tolerance group (F (1,20) = 6.72, $p < .05$), but not for the rats from the Control group (F (1,8) = 0.3, $p > .90$.

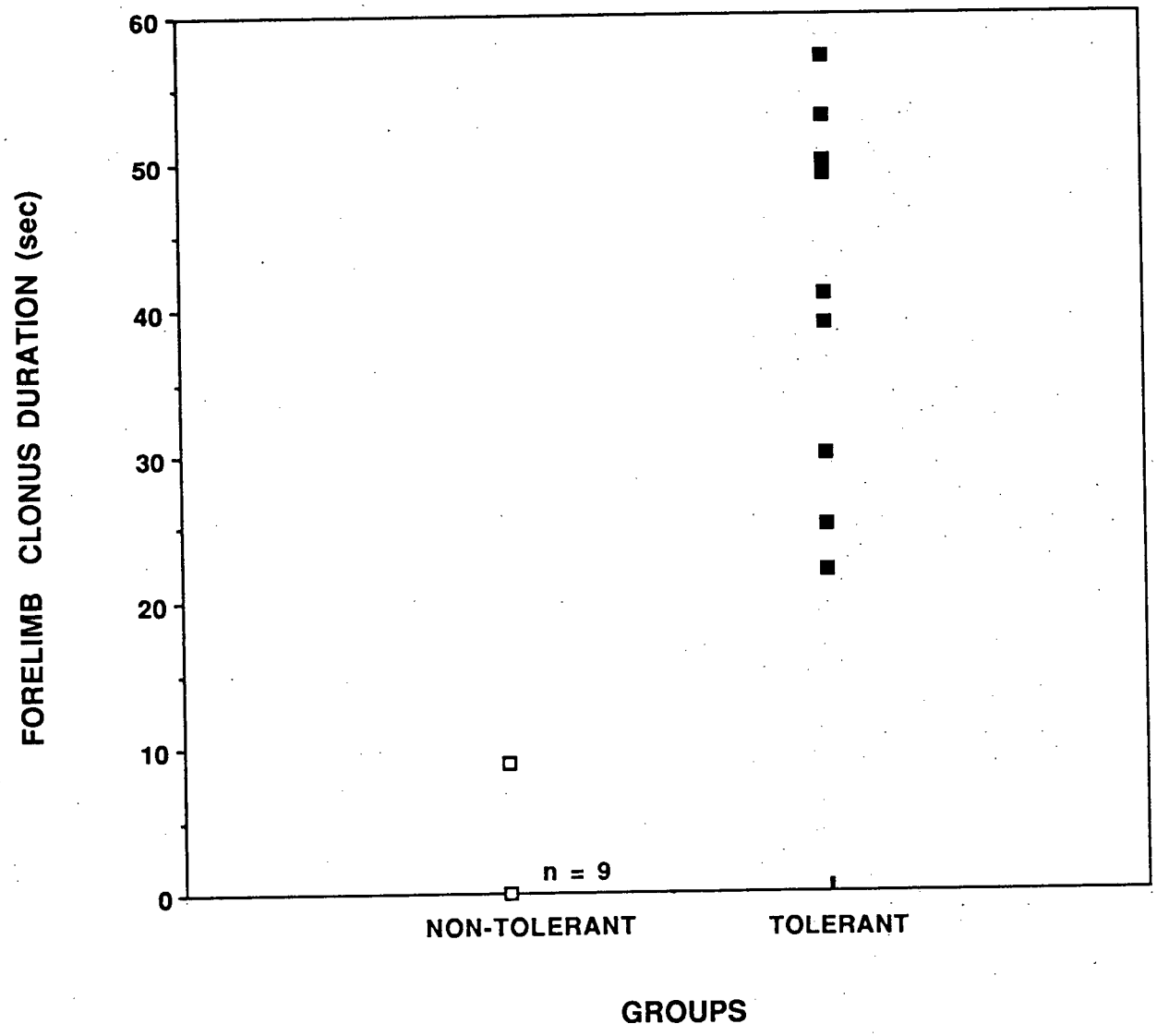
Discussion

The results of Experiment 3 support the many previous demonstrations of pharmacologic tolerance to DZP's anticonvulsant effect, including the report by Löscher and Schwark (1985) of pharmacologic tolerance to DZP's anticonvulsant effect on kindled convulsions in the rat. It is interesting to note that Löscher and Schwark (1985) reported only a modest amount of tolerance in the rats from their experiment, an observation that was supported by the initial analysis of the data from the present experiment. Thus, a comparison between the magnitude of pharmacologic tolerance to DZP's anticonvulsant effect presented in Figure 10 in the present experiment and the magnitude of contingent tolerance to DZP's anticonvulsant effect presented in Figure 8 from Experiment 2 leads to the conclusion that the development of tolerance is more complete in kindled rats that receive convulsive stimulation during periods of drug exposure. However, this conclusion cannot be supported by the data from these two

experiments; when the data for the rats from the Pharmacologic-Tolerance group that actually developed tolerance to DZP's anticonvulsant effect were compared to the data from the rats from the Contingent-Tolerance group from Experiment 2, it is clear that the magnitude of tolerance is almost equal for the two groups (Mean = 35.3 for the Contingent-Tolerance rats from Experiment 2 compared to Mean = 39.9 for the Pharmacologic-Tolerance rats in the present experiment). The apparent difference in magnitude between contingent tolerance and pharmacologic tolerance is actually due to the fact that fewer rats in a given group develop pharmacologic tolerance than contingent tolerance to DZP's anticonvulsant effect; that is, although half of the rats (11/21) in the Pharmacologic-Tolerance group displayed a substantial degree of tolerance to DZP's anticonvulsant effect, the other half of the rats in this group displayed no tolerance at all. This dispersion in the degree of tolerance development in the rats from the Pharmacologic-Tolerance group in Experiment 3 is illustrated in Figure 11.

The remaining experiments explore the question of whether pharmacological and contingent tolerance to DZP's anticonvulsant effect are attributable to a single set of physiological changes or instead are expressions of independent changes in the nervous system. In the next experiment, the rate of dissipation of these two phenomenologically distinct forms of tolerance was examined to determine whether there was any difference in the reversal of

Figure 11. Distribution of data for the rats from the Pharmacologic-Tolerance group on the tolerance-test trial. DZP continued to exert an anticonvulsant effect on 10 rats from the Pharmacologic-Tolerance group (as noted, 9 of the rats in this group displayed no forelimb clonus on the tolerance-test trial), but tolerance had clearly developed in the other 11 rats in this group.


FORELIMB CLONUS DURATION (sec)
60
50
40
30
20
10
0
n = 9
NON-TOLERANT
TOLERANT
GROUPS

the physiological changes responsible for their development and expression.

VII. Experiment 4

A difference in the rate at which tolerance to a drug's effects develops or dissipates has been assumed to reflect a difference in the physiological changes that underlie the development of tolerance (e.g., File, 1985; LeBlanc et al., 1976; Okamoto, 1984; Teitz & Rosenberg, 1988). For example, LeBlanc et al (1976) argued that the fact that contingent tolerance and pharmacologic tolerance to ethanol's ataxic effect dissipated at the same rate indicated that these two forms of tolerance were separate manifestations of a single set of underlying physiological changes. And Teitz and Rosenberg (1988), using a similar form of logic, proposed that the fact that the development and dissipation of tolerance to the anticonvulsant effects of the benzodiazepine flurazepam occurs much more slowly than that for the drug's locomotor effects reflected a difference in the physiological bases of these two forms of tolerance.

In Experiment 4, this comparative-rate approach was used in an effort to determine whether pharmacologic and contingent tolerance to DZP's anticonvulsant effect are attributable to a single physiological change or instead to unique physiological changes in the nervous system. Experiment 4 focused upon the rate of dissipation of pharmacologic and contingent tolerance to

DZP's anticonvulsant effect.

Methods

Subjects. The subjects were 151 male, Long-Evans rats (Charles River, Canada), weighing between 350 g and 400 g at the time of surgery and between 500 g and 650 g at the completion of the experiment.

Drugs. DZP (2 mg/kg, IP) was injected at a volume of 2 ml/kg in a 2% Tween-80/ isosaline vehicle.

Kindling Phase. A total of 16 rats were lost during the kindling phase; 10 rats did not meet the criterion for kindling and the electrode assemblies became detached in 6 others.

Stimulation-Baseline Phase. The stimulation-baseline phase began 48 hr after the completion of the kindling phase. Rats that did not demonstrate at least 20 sec of forelimb clonus on the last stimulation-baseline trial were not studied further (n=4).

Drug-Baseline Trial. The drug-baseline trial occurred 48 hr after the fourth and last stimulation-baseline trial. On the drug-baseline trial, each rat received DZP 1 hr before the convulsive stimulation. Rats not showing at least an 80% decrease in forelimb clonus duration on the drug-baseline trial relative to the last trial of the stimulation-baseline phase were not studied further (n=21). The remaining 110 rats were assigned to one of four groups--a Pharmacologic-Tolerance group (n=57), a Pharmacologic-Control group (n=9), a Contingent-Tolerance group (n=36), or a Contingent-Control group (n=8)--so that the mean

forelimb clonus durations on both the last stimulation-baseline trial and the drug-baseline trial were approximately equal for each group.

Tolerance-Development Phase. The tolerance-development phase for the rats from the Pharmacologic-Tolerance and the Pharmacologic-Control groups began 24 hr after the drug-baseline trial. During the 10-day tolerance-development phase, the rats were not stimulated. The rats in the Pharmacologic-Tolerance group received DZP every 8 hr for 10 days; the rats from the Pharmacologic-Control group received vehicle injections on the same schedule.

The tolerance-development phase for the rats from the Contingent- Tolerance and the Contingent-Control groups began 48 hr after the drug-baseline trial. The rats in both groups continued to received a convulsive stimulation every 48 hr during the 20-day tolerance-development phase. Each rat received an injection 1 hr before each stimulation; the rats from the Contingent-Tolerance group received DZP, whereas the rats from the Contingent-Control group received an equal volume of the vehicle.

Tolerance-Test Trial. The tolerance-test trial occurred 8 hr after the last injection of the tolerance-development phase for the rats from the Pharmacologic-Tolerance and the Pharmacologic-Control groups and 48 hr after the last tolerance-development trials for the rats from the Contingent-Tolerance and the

Contingent-Control groups. The tolerance-test trial was identical for all of the rats; an injection of DZP was administered 1 hr before convulsive stimulation and the duration of forelimb clonus elicited by the stimulation was recorded.

Following the tolerance-test trial, rats from the Pharmacologic-Tolerance and the Contingent-Tolerance groups that did not demonstrate at least 20 sec of forelimb clonus on the tolerance-test trial were removed from the experiment (n = 21 for the Chronic-Tolerance group; n = 4 for the Contingent-Tolerance group). Accordingly, by the end of the tolerance-test trial the 85 rats that remained in the experiment (Pharmacologic-Tolerance group n = 36; Pharmacologic-Control n = 9; Contingent-Tolerance group n = 32; Contingent-Control n = 8) were all tolerant to DZP's anticonvulsant effect.

The tolerant rats in both the Pharmacologic-Tolerance and Contingent-Tolerance groups were then assigned to one of four tolerance-retention groups, which determined whether they were retested 2 days, 4 days, 8 days, or 16 days after the tolerance-test trial. The rats from the Pharmacologic-Control and Contingent-Control groups were retested only at the 16-Day interval. Thus, there were 10 groups of rats in Experiment 4: These were the Pharmacologic-Tolerance 2-Day, 4-Day, 8-Day, 16-Day, and Control groups; and the Contingent-Tolerance 2-Day, 4-Day, 8-Day, 16-Day, and Control groups.

Tolerance-Retention Interval. During the tolerance-retention

interval, each rat was remained in its home cage until it was scheduled to be retested.

Tolerance-Retention Test-Trial. The protocol followed on the tolerance-retention test-trial for the rats from the Pharmacologic-Tolerance and the Contingent-Tolerance groups was identical to the drug-baseline trial and the tolerance-test trial; on the appropriate day after the tolerance-test trial, DZP was administered 1 hr before a convulsive stimulation was delivered and the duration of forelimb clonus elicited by the stimulation was recorded.

The rats from the two Control groups were not retested until 16 days after the tolerance-test trial. The nature of the treatment that the rats from the two Control groups received on the tolerance-retention test-trial was determined by the performance of the corresponding Pharmacologic-Tolerance and Contingent-Tolerance groups on the 16-Day Retention trial. The rats from the Pharmacologic-Tolerance 16-Day group showed a substantial loss of tolerance to DZP's anticonvulsant effect; consequently, the rats from the Pharmacologic-Control group received a vehicle injection 1 hr before convulsive stimulation was delivered on the tolerance-retention test trial. The purpose of stimulating these control rats in an undrugged state was to control for the possibility that the loss of tolerance to DZP's anticonvulsant effect in the rats from the Pharmacologic-Tolerance 16-Day group was due to a decline in the sensitivity of

the rats to the kindling stimulation rather than to a genuine dissipation of tolerance. In contrast, there was no loss of tolerance in the rats from the Contingent-Tolerance 16-Day group. Consequently, the rats from the Contingent-Control group were administered DZP 1 hr before they were stimulated on the tolerance-retention test trial. The purpose of stimulating these rats in a drugged state was to determine whether the retention of tolerance to DZP's anticonvulsant effect displayed by the rats from Contingent-Tolerance group was genuine, or instead attributable to an increase in the sensitivity of the rats to the convulsive stimulation over the 16-day retention interval.

Statistics. A single 10 (Groups) X 2 (Trials), between-within factor, repeated-measures analysis of variance was used to analyze the data from the tolerance-test trial and the tolerance-retention-trial for the five Pharmacologic-Tolerance groups and the five Contingent-Tolerance groups in Experiment 4. Tests of simple main effects were performed on the respective between- and within group factors involved in the significant interaction; where necessary, Neuman-Keuls posthoc comparisons were used to further analyze the data from significant tests of simple main effects.

Results.

The retention of pharmacologic and contingent tolerance to DZP's anticonvulsant effect over the 16-day retention interval is presented in Figure 12 (Pharmacologic Tolerance) and Figure 13

(Contingent Tolerance). As in the previous experiments, the test dose of DZP almost completely suppressed the forelimb clonus in the rats from each of the 10 treatment groups on the drug-baseline-trial. In contrast, on the tolerance-test trial the rats from the four Pharmacologic-Tolerance and four Contingent-Tolerance groups that were included in the final analysis displayed a substantial degree of tolerance to the anticonvulsant effect of DZP during the tolerance-development phase. Over the 16-day retention interval, pharmacologic tolerance to DZP's anticonvulsant effect gradually dissipated; the test dose of DZP completely suppressed the forelimb clonus of almost every rat in the Pharmacologic-Tolerance 16-day group (see Figure 12). In contrast, there was no evidence of a decline in contingent tolerance to DZP's anticonvulsant effect over the 16-day retention interval; there was little difference in the mean duration of forelimb clonus between the tolerance-test trial and the tolerance-retention-test trial for the rats from the Contingent-Tolerance 16-Day group (see Figure 13).

The statistical analyses support the observations made from Figure 12 and Figure 13. The analysis of variance revealed a significant group X trial interaction ($\underline{F}$ (9,75) = 7.37, $\underline{p}$ <.001). The analyses of simple main effects for each group across the tolerance-test and retest-trials revealed a significant decrease in forelimb clonus for the rats from the Pharmacologic-Tolerance 16-Day group ($\underline{F}$ (1,7) = 81.86, $\underline{p}$ < .001), but not for any of the

Figure 12. Dissipation of pharmacologic tolerance to DZP's anticonvulsant effect. DZP had a potent anticonvulsant effect on all of the rats on the drug-baseline trial; in contrast, on the tolerance-test trial the drug's anticonvulsant effect had almost disappeared for the four Pharmacologic-Tolerance groups. There was a steady loss of tolerance to DZP's anticonvulsant effect over the retention interval, although there was a significant difference between the tolerance-test and retest trial only for the rats from the 16-Day group. This loss of tolerance cannot be attributed to a loss of sensitivity to the stimulation over the retention interval because the rats from the Control group displayed as much forelimb clonus on the retention-test trial as they had on the last trial of the stimulation-baseline phase.

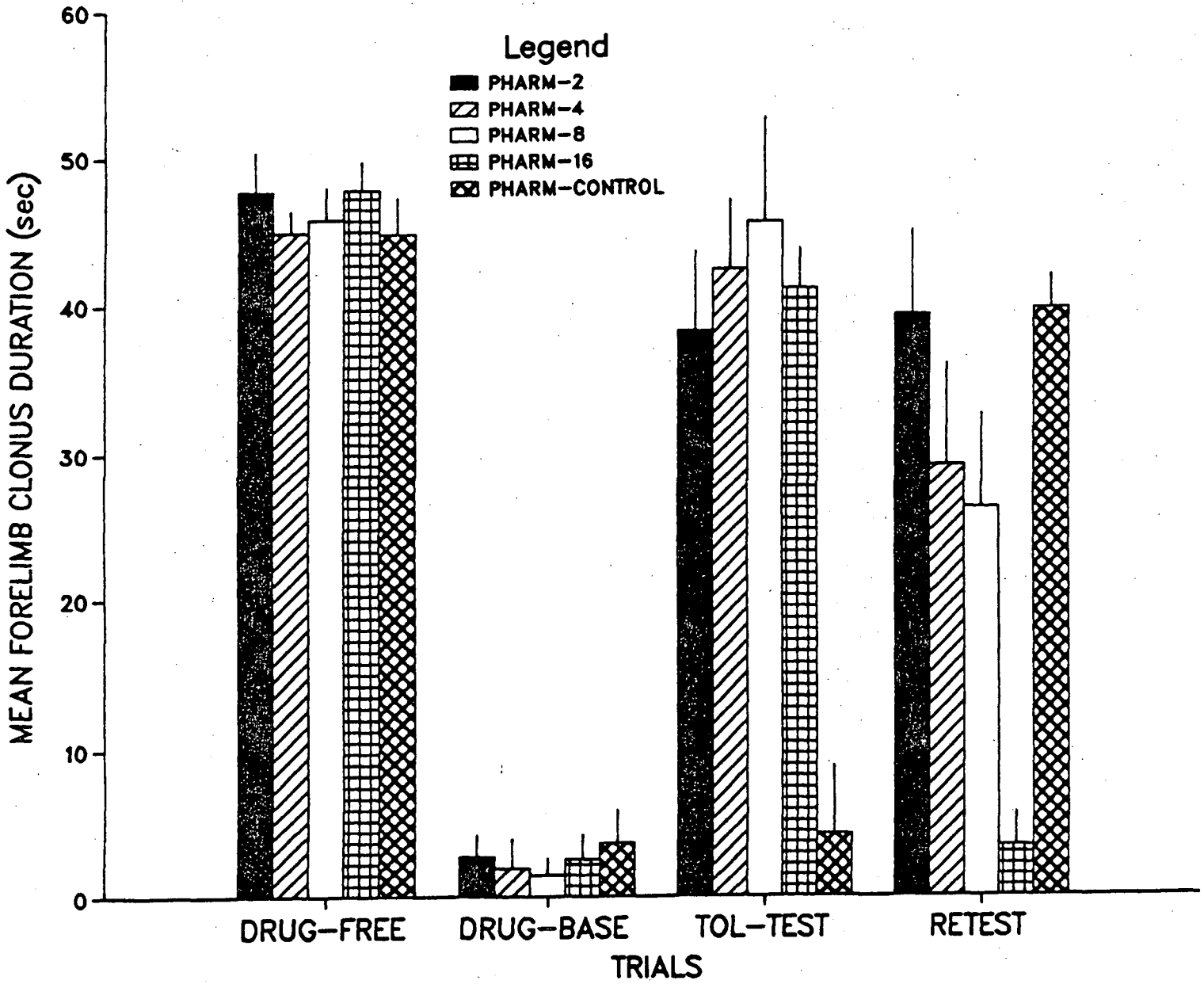
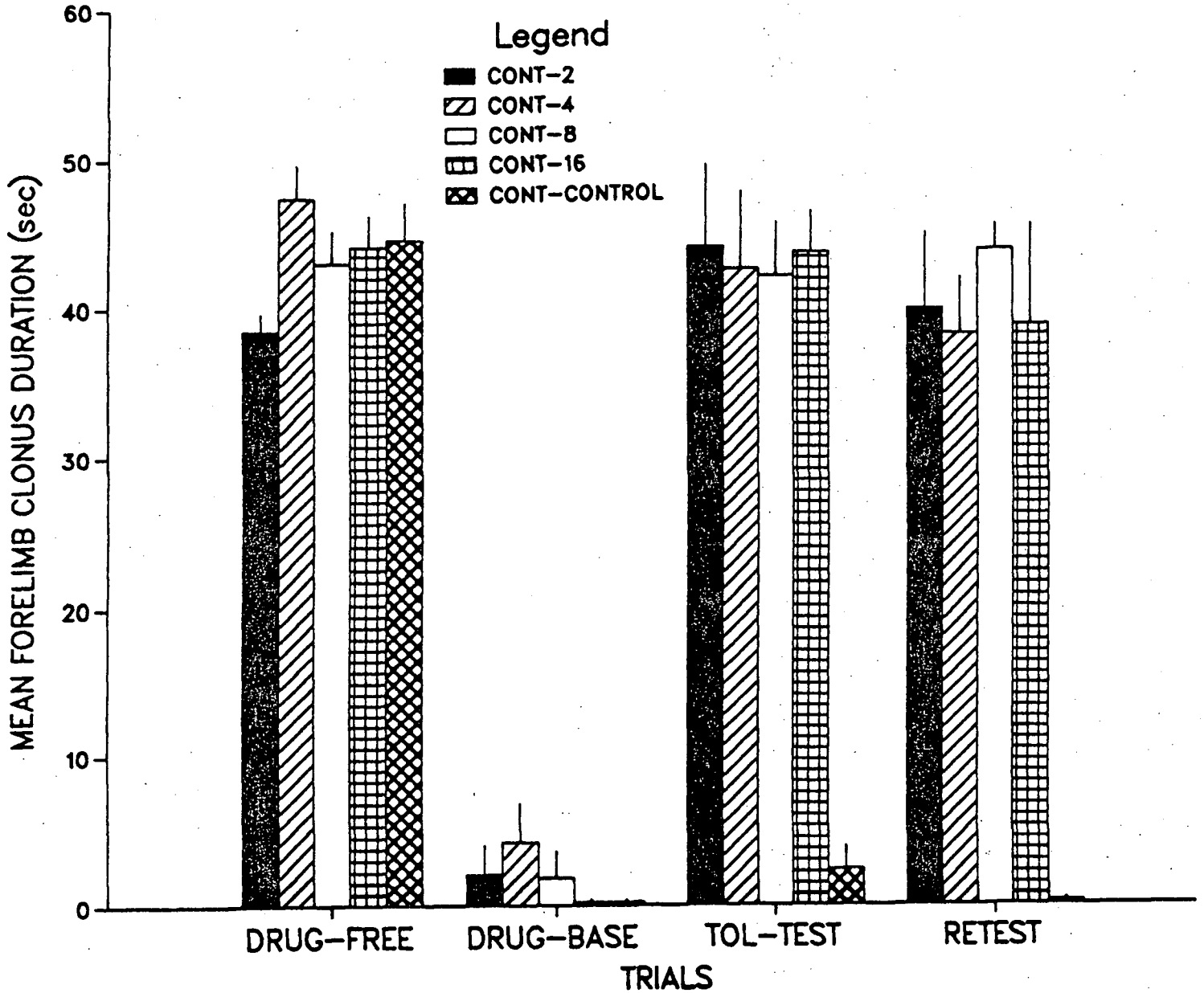

Legend
PHARM-2
PHARM-4
PHARM-8
PHARM-16
PHARM-CONTROL
MEAN FORELIMB CLONUS DURATION (sec)
60
50
40
30
20
10
0
DRUG-FREE
DRUG-BASE
TOL-TEST
RETEST
TRIALS

Figure 13. Dissipation of contingent tolerance to DZP's anticonvulsant effect. DZP had a potent anticonvulsant effect on all of the rats on the drug-baseline trial; in contrast, on the tolerance-test trial the drug's anticonvulsant effect had almost disappeared in the four Contingent-Tolerance groups. There is no evidence of a loss of tolerance to DZP's anticonvulsant effect over the retention interval; there was no significant difference between the tolerance-test trial and the retention-test trial for any of the four Contingent-Tolerance groups. This cannot be attributed to an increase in the sensitivity of these rats to the stimulation over the retention interval as DZP continued to suppress the convulsions of the rats from the Contingent-Control group on the retest trial.


Legend
CONT-2
CONT-4
CONT-8
CONT-16
CONT-CONTROL
MEAN FORELIMB CLONUS DURATION (sec)
60
50
40
30
20
10
0
DRUG-FREE
DRUG-BASE
TOL-TEST
RETEST
TRIALS

other Pharmacologic-Tolerance groups or any of the Contingent-Tolerance groups (all p's > .10). The analyses for simple main effects across groups within a trial revealed a significant effect for both the tolerance-test trial (F (9,75) = 7.55, p < .001) and the tolerance-retest trial (F (9,75) = 6.48, p < .001). Further analysis of the data from the tolerance-test trial with Neuman-Keuls posthoc comparisons indicated that the rats from the two Control groups displayed significantly less tolerance than any of the other groups on the tolerance-test trial (all Neuman-Keuls p's < .05), and there were no differences between the different Pharmacologic-Tolerance and Contingent-Tolerance groups on this trial (all Neuman-Keuls p's > .10). Neuman-Keuls posthoc analysis of the data from test of simple main effects for the tolerance-retest trial indicated that the rats from the Pharmacologic-Tolerance 4-Day, 8-Day, and 16-Day groups, and the Contingent-Control group, displayed signficantly less forelimb clonus than the rats from the Pharmacologic-Tolerance 2-Day group and all four of the Contingent-Tolerance groups (all Neuman-Keuls p's < .05).

Discussion

The difference in the rate of dissipation between pharmacologic tolerance and contingent tolerance to DZP's anticonvulsant effect supports the idea that these phenomenologically distinct forms of tolerance also represent independent physiological changes. It is particularly

interesting to note that the time course for the dissipation of pharmacologic tolerance (8 days) in the present experiments is similar to that reported by Teitz and Rosenberg (1988) for the dissipation of tolerance to DZP's anticonvulsant effects on PTZ convulsions (between 4 and 7 days). This finding gains further signficance when considered in light of the observation by Gonsalves and Gallager (1987) that the impairment of GABAergic function produced by chronic DZP administration also dissipates within 8 days of the cessation of drug exposure. Based upon these observations, Gonsalves and Gallager (1988) and Teitz and Rosenberg (1988) have suggested that a downregulation in the number of benzodiazepine receptors (which occurs rapidly when benzodiazepines are administered on a chronic basis and disappears rapidly when the drug is withdrawn) underlies the development of tolerance to DZP's locomotor effects, whereas a decrease in the sensitivity of GABA-A receptors associated with benzodiazepine receptors (which occurs slowly when benzodiazepines are administered on a chronic basis and disappears slowly when the drug is withdrawn) underlies the development of tolerance to the drug's anticonvulsant effects.

If this hypothesis is correct, the fact that the time course for the dissipation of contingent tolerance to DZP's anticonvulsant effect is substantially different from that for the dissipation of pharmacologic tolerance to DZP's anticonvulsant effect and the dissipation of the GABAergic

subsensitivity that results from chronic DZP exposure raises the possibility that the development of contingent tolerance to DZP's anticonvulsant effect is not attributable to a decrease in the sensitivity of GABA-A receptors associated with benzodiazepine receptors. This possibility is examined in Experiment 5.

VIII. Experiment 5

The discovery of the benzodiazepine receptor antagonist RO 15-1788 by Hunkeler et al. (1981) provided an important pharmacologic tool for studying the structure and function of the benzodiazepine receptor. Significantly less toxic than most of the classic benzodiazepine agonists (e.g., LD50 of 1,360 mg/kg, IP, in the rat; Hunkeler et al., 1981), RO 15-1788 was originally reported to be devoid of any of the behavioral effects associated with the classic benzodiazepines such as DZP; Hunkeler et al. (1981) reported no evidence of significant sedative, anticonvulsant, muscle relaxant, or anxiolytic effects in either mice, rats, cats or dogs--even at near-toxic doses. In addition, RO 15-1788 did not appear to be a proconvulsant or to produce stimulatory effects in these species (see Hunkeler et al., 1981).

Since the seminal report by Hunkeler and his colleagues, it has become apparent that RO 15-1788 is not behaviorally or physiologically inert. RO 15-1788 has a variety of intrinsic effects that can complement or oppose those of the classical benzodiazepine agonists in a dose- and test-dependent manner (see

File & Pellow, 1986, for a review of this literature). Of particular significance to the present experiments, Gonsalves and Gallager (1988) found that a single injection of RO 15-1788, administered 24 hr prior to testing, reverses both the subsensitivity of benzodiazepine-linked GABA-A receptors that is produced by chronic benzodiazepine treatment and the expression of pharmacologic tolerance to the anticonvulsant effects of DZP on bicuculline-induced convulsions in rats. The results of Experiment 4 suggested that a decrease in the sensitivity of the GABA-A receptor might be responsible for the development of pharmacologic tolerance, but not contingent tolerance, to DZP's anticonvulsant effect on kindled convulsions. If this is true, then a single injection of RO 15-1788 should subsequently reduce the expression of pharmacologic tolerance, but not contingent tolerance, to DZP's anticonvulsant effect on kindled convulsions. The purpose of Experiment 5 was to test this hypothesis.

Methods

Subjects. The subjects were 51 male, Long-Evans rats, weighing between 350 g and 400 g at the time of surgery and between 500 g and 650 g at the completion of the experiment.

Drugs. DZP (2 mg/kg) was injected at a volume of 2 ml/kg in a 2% Tween-80/isosaline vehicle.

Kindling Phase. Four rats that did not meet the criterion for kindling were removed from the experiment at the end of this phase.

Stimulation-Baseline Phase. The stimulation-baseline phase began 48 hr after the completion of the kindling phase. All of the remaining rats successfully completed the stimulation-baseline phase.

Drug-Baseline Trial. The drug-baseline trial occurred 48 hr after the fourth and last stimulation-baseline trial. On the drug-baseline trial, each rat received DZP 1 hr before the convulsive stimulation. Rats not showing at least an 80% decrease in forelimb clonus duration on the drug-baseline trial relative to the last trial of the stimulation-baseline phase were not studied further (n = 4). The remaining 43 rats were assigned to one of four groups--a Pharmacologic-Tolerance group (n = 14), a Pharmacologic-Control group (n = 10), a Contingent-Tolerance group (n = 10), and a Contingent-Control group (n = 9)--so that the mean forelimb clonus durations on both the last stimulation-baseline trial and the drug-baseline trial were approximately equal for the four groups.

Tolerance-Development Phase. The tolerance-development phase for the rats from the Pharmacologic-Tolerance and the Pharmacologic-Control groups began 24 hr after the drug-baseline trial. During the 10-day tolerance-development phase, the rats were not stimulated. The rats in the Pharmacologic-Tolerance group received DZP every 8 hr for 10 days; the rats from the Pharmacologic-Control group received vehicle injections on the same schedule.

The tolerance-development phase for the rats from the Contingent- Tolerance and the Contingent-Control groups began 48 hr after the drug-baseline trial. The rats in both groups continued to received convulsive stimulation every 48 hr during the 20-day tolerance-development phase. Each rat received an injection 1 hr before each stimulation; the rats from the Contingent-Tolerance group received DZP, whereas the rats from the Contingent-Control group received an equal volume of the vehicle.

Tolerance-Test Trial. The tolerance-test trial occurred 8 hr after the last injection of the tolerance-development phase for the rats from the Pharmacologic-Tolerance and the Pharmacologic-Control groups and 48 hr after the last tolerance-development trial for the rats from the Contingent-Tolerance and the Contingent-Control groups. The tolerance-test trial was identical for all of the rats; an injection of DZP was administered 1 hr before convulsive stimulation and the duration of forelimb clonus elicited by the stimulation was recorded.

Following the tolerance-test trial, rats from the Pharmacologic- Tolerance and the Contingent-Tolerance groups that did not demonstrate at least 20 sec of forelimb clonus on the tolerance-test trial were removed from the experiment ($n = 5$ for the Chronic-Tolerance group; $n = 2$ for the Contingent-Tolerance group). Accordingly, by the end of the tolerance-test trial 36 rats that remained in the experiment (Pharmacologic-Tolerance

group n = 9; Pharmacologic-Control group, n = 10; Contingent-Tolerance group n = 8; Contingent-Control group, n = 9).

RO 15-1788 Administration. A single injection of RO 15-1788 (5 mg/kg, IP, in a 2% Tween 80/isosaline solution at a volume of 2 ml/kg) was administered to each rat 24 hr after the tolerance-test trial.

Tolerance-Retest Trial. The tolerance-retest trial occurred 24 hr after the adminstration of RO 15-1788. The protocol followed on the tolerance-retest trial for the rats from the Pharmacologic-Tolerance and Contingent-Tolerance groups was identical to the drug-baseline trial and the tolerance-test trial; the test dose of DZP was administered 1 hr before convulsive stimulation was delivered and the duration of forelimb clonus elicited by the stimulation was recorded. In contrast, the rats from the two Control groups received an injection of the vehicle 1 hr before they were stimulated; the purpose of this procedure was to control for the possibility that RO 15-1788 might affect the expression of tolerance to DZP's anticonvulsant effect by affecting the sensitivity of the kindled rats to the convulsive stimulation.

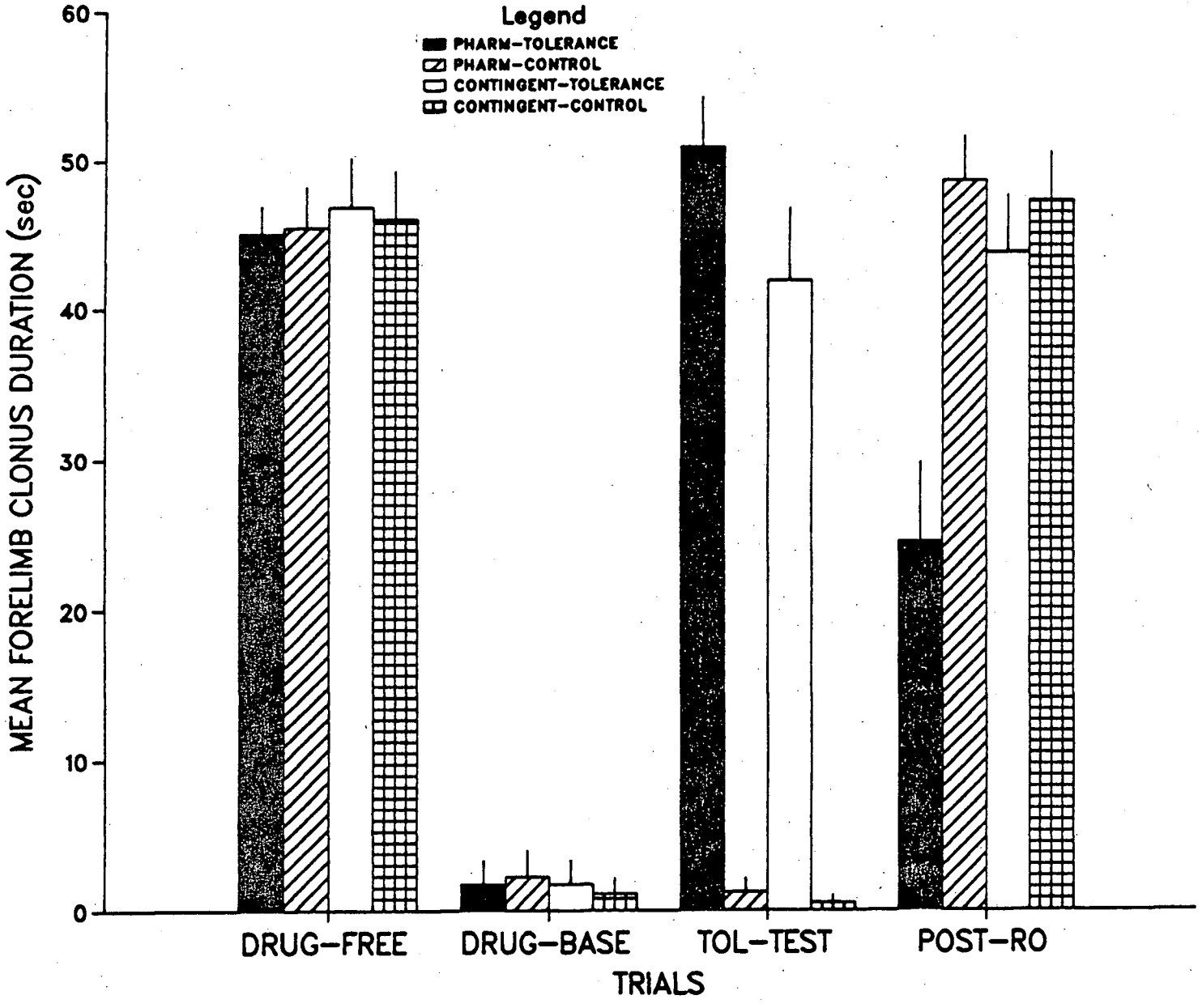
Statistics. A single 4 (Groups) X 2 (Trials) between-within factors, repeated-measures analysis of variance was used to analyze the data from the tolerance-test trial and the tolerance-retest trial of Experiment 5. Tests of simple main effects were used to assess the contribution of the respective between- and

within-groups factors to the significant interaction; where necessary, Neuman-Keuls posthoc comparisons were used to further analyze the data from significant tests of simple main effects. Finally, separate correlated t-tests were used to analyze the data from the last stimulation-baseline trial and the retest trial for the rats from the two control groups.

Results

The effects of RO 15-1788 on the expression of pharmacologic and contingent tolerance to DZP's anticonvulsant effect on kindled convulsions are illustrated in Figure 14. The test dose of DZP had a potent anticonvulsant effect on almost all of the rats on the drug-baseline trial. On the tolerance-test trial, the rats from the Pharmacologic-Tolerance and Contingent-Tolerance groups displayed a substantial amount of tolerance to DZP's anticonvulsant effect, whereas there was no evidence of tolerance in the rats from the two Control groups. It is clear that RO 15-1788 had little effect on the expression of contingent tolerance to DZP's anticonvulsant effect; there was no difference in the mean duration of forelimb clonus between the tolerance-test trial and the tolerance-retest trial for the rats from the Contingent-Tolerance group. In contrast, RO 15-1788 had a significant effect on the expression of tolerance in the rats from the Pharmacologic-Tolerance group on the tolerance-retest trial; the mean duration of forelimb clonus for the rats from

Figure 14. Effects of RO 15-1788 on the dissipation of pharmacologic and contingent tolerance to DZP's anticonvulsant effect. DZP almost completely suppressed forelimb clonus in each rat on the drug-baseline trial. In contrast, on the tolerance-test trial the rats from the Pharmacologic-Tolerance and Contingent-Tolerance groups displayed a considerable amount of tolerance to DZP's anticonvulsant effect. The RO 15-1788 was administered to each rat between the tolerance-test trial and the tolerance-retest trial; it reduced the expression of tolerance in the rats from the Pharmacologic-Tolerance group but had no effect on the tolerance in the Contingent-Tolerance group or on the sensitivity of the two control groups to the convulsive stimulation.


Legend
PHARM–TOLERANCE
PHARM–CONTROL
CONTINGENT–TOLERANCE
CONTINGENT–CONTROL
MEAN FORELIMB CLONUS DURATION (sec)
60
50
40
30
20
10
0
DRUG–FREE
DRUG–BASE
TOL–TEST
POST–RO
TRIALS

this group decreased over 26 sec (52%) between the tolerance-test trial and tolerance-retest trial. This effect could not be attributed to an RO 15-1788 induced decrease in the sensitivity of the rats from the Pharmacologic-Tolerance group to the convulsive stimulation because the drug had no effect on the duration of forelimb clonus of the rats from either the Pharmacologic-Control group or the Contingent-Control group on the tolerance-retest trial.

The statistical analysis of the data from Experiment 5 confirms the observations made from Figure 14. There was a significant group X trial interaction (F (3,32) = 72.01, p < .01). The tests of simple main effects revealed a significant decrease on the tolerance-retest trial compared to the tolerance-test trial for the rats from the Pharmacologic-Tolerance group (F (1,8) = 16.96, p < .01), but not for the rats from the Contingent-Tolerance group (F (1,7) = 0.25, p > .50). Tests of simple main effects revealed a significant difference between groups on both the tolerance-test trial (F (3,32) = 39.7, p < .0001) and the tolerance-retest trial (F (3,32) = 7.12, p < .01). Neuman-Keuls posthoc analysis of this test of simple main effects across groups on the tolerance-test trial indicated that the rats from the two control groups displayed less forelimb clonus than the rats from the Pharmacologic-Tolerance and Contingent-Tolerance groups (all Neuman-Keuls p's <.05); there were no differences between the two control groups (Neuman-Keuls p > .05)

or the Pharmacologic-Tolerance and the Contingent-Tolerance groups (Neuman-Keuls $p > .05$). Neuman-Keuls analysis of the test of simple main effects across groups on the tolerance-retest trial indicated that the rats from the Pharmacologic-Tolerance group displayed significantly less forelimb clonus than the rats from the Contingent-Tolerance group or the two control groups (all p's $< .05$), and there were no other differences between these latter 3 groups (all p's $> .05$). Correlated t-tests revealed no differences between the last stimulation-baseline trial and the retest trial for either of the control groups, both p's $> .10$.

Discussion

The reduction of pharmacologic tolerance to DZP's anticonvulsant effect on kindled convulsions by a single injection of RO 15-1788 extends Gonsalves and Gallager's earlier (1987) finding that RO 15-1788 reduces the expression of pharmacologic tolerance to DZP's anticonvulsant effect on bicuculline-induced convulsions in mice. Although RO 15-1788 has been shown to possess limited anticonvulsant properties (see File & Pellow, 1986), it is unlikely that this property was responsible for its effects on the expression of pharmacologic tolerance to DZP's anticonvulsant effect because the dose of RO 15-1788 had no anticonvulsant effect on the convulsions of the rats in the two control groups. In addition, Lister, Greenblatt,

Abernethy, and File (1984) have shown that RO 15-1788 has a half life in the CNS of about 16 min following an IP injection (at a dose of 10 mg/kg, IP); it is therefore unlikely that RO 15-1788 would even be present in the brain 24 hr after its administration, which is when the convulsive stimulation for the tolerance-retest trial was delivered in Experiment 5.

The demonstration that RO 15-1788 reverses pharmacologic tolerance to DZP's anticonvulsant effect on kindled convulsions provides further support for the hypothesis that this form of tolerance is attributable to a decrease in the sensitivity of GABA-A receptors (see also Gonsalves & Gallager, 1985; 1987). Furthermore, the fact that an identical dose of RO 15-1788 had no effect on the expression of contingent tolerance to DZP's anticonvulsant effect strengthens the claim that the physiological changes which underlie the development of contingent tolerance are not the same as those responsible for the development of pharmacologic tolerance to DZP's anticonvulsant effect. Meldrum and Chapman (1986) have previously suggested that different physiological changes underlie the development of tolerance to DZP's anticonvulsant effect on maximal electroshock convulsions and pentylenetetrazol convulsions, respectively. However, the present results are unique because they indicate that different physiological changes may underlie the development of tolerance to the same effect, of the same drug, on the same type of convulsion--with the nature of

the physiological change dependent upon the schedule of drug administration and/or the administration of convulsive stimulation during periods of drug exposure.

Although the present results support the idea that separate physiological changes are responsible for pharmacologic and contingent tolerance to DZP's anticonvulsant effect, they do not provide incontrovertible evidence for this conclusion. For example, RO 15-1788 may have antagonized contingent tolerance to DZP's anticonvulsant effect if a larger dose was used in the present experiment. Alternatively, contingent tolerance to DZP's anticonvulsant effect may result from a physiological change completely independent of the GABA-A/benzodiazepine receptor complex. If this is the case, the administration of RO 15-1788 would not affect the expression of contingent tolerance to DZP's anticonvulsant effect, regardless of the dose of the antagonist that was administered. This issue is examined more fully in the general discussion.

IX. GENERAL DISCUSSION.

The results of the five experiments reported in the present dissertation lead to two general conclusions. First, they clearly indicate that the occurrence of convulsive stimulation during periods of drug exposure can play a key role in the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA. And second, they support the idea that contingent tolerance and pharmacologic tolerance, at least to the anticonvulsant effect of DZP, are physiologically distinct. These two general conclusions are discussed in more detail in the following two major sections of this general discussion.

1. The Role of Convulsive Stimulation in the Development of Contingent Tolerance to the Anticonvulsant Effects of CBZ, DZP, and VPA.

The most notable feature of the results from Experiment 1 was the magnitude of the tolerance that developed to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions in the rat. Previous attempts to study the development of tolerance to the anticonvulsant effects of these drugs on kindled convulsions had reported only a moderate level of tolerance for CBZ (Hönack & Löscher, 1988) and DZP (Löscher & Schwark, 1985), and a complete lack of tolerance to VPA's anticonvulsant effects on kindled convulsions (Young et al., 1987) of tolerance. In contrast, the magnitude of the tolerance that developed to the anticonvulsant effects of CBZ, DZP, and VPA

on kindled convulsions in Experiment 1 was considerably greater; in fact, tolerance developed to such an extent that there was little difference in the durations of forelimb clonus between the tolerance-test trial and the last trial of the no-drug baseline phase for any of the three drug groups in this experiment.

Several possible explanations for the magnitude of the tolerance reported in Experiment 1 were considered. However, previous work from our laboratory (e.g., Pinel et al., 1983; 1985; 1989) indicated that the convulsive stimulation that the rats in each of the three drug groups in Experiment 1 received on each tolerance-development trial 1 hr after the appropriate drug was administered was likely an important factor in the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA. The results of Experiment 2 clearly supported this idea. In Experiment 2, tolerance developed rapidly to the anticonvulsant effects of CBZ, DZP, and VPA in the kindled rats from each of the three drug-before-stimulation groups; in contrast, there was little evidence of tolerance development in the kindled rats from the three drug-after-stimulation groups or the three vehicle-control groups.

The fact that there have been no previous reports that seizure activity during periods of drug exposure can influence the development of tolerance to the anticonvulsant effects of antiepileptic drugs is especially interesting given the existence of several anecdotal reports that support such an idea. For example, Killam et al. (1973) reported that the development of

tolerance to diazepam's anticonvulsant effect on photically induced seizures in the baboon P. papio could be prevented if a relatively high treatment dose was used from the start of treatment. This observation was interesting for two reasons. First, the fact that a high treatment dose retarded the development of tolerance contradicts the traditional assumption that the development of tolerance is facilitated by the administration of high treatment doses (e.g., Kalant et al., 1971). Second, the use of a high treatment dose conflicts with the normal clinical procedure, noted earlier, to initiate pharmacologic treatment of epilepsy with as low a dose as possible and to increase it only when a loss of efficacy is noted (e.g., Eadie, 1985; note that this treatment strategy, though advocated as a way to reduce the incidence of unwanted side effects, also reflects the traditional assumption that the development of tolerance is facilitated by the administration of a high treatment dose). Although the data reported by Killam et al. (1973) are puzzling when viewed from the traditional perspective of pharmacologic tolerance, they can be explained from the perspective of contingent tolerance--the use of a high treatment dose would reduce the incidence of seizure activity in P. papio, thereby reducing the facilitatory effect that the occurrence of such activity during periods of drug exposure could have on the development of tolerance.

The idea that convulsive stimulation during periods of drug exposure might facilitate the development of tolerance to the

anticonvulsant effect of antiepileptic drugs is also supported by Voskuyl et al. (1986), who found that tolerance developed to the anticonvulsant effects of diazepam (2.5 mg/kg, IP) on maximal electroshock seizures only when the rats received convulsive stimulation 30 min after each DZP injection. There was no evidence of tolerance in rats that received a "minimum" number (no other details given) of convulsive stimulations during the tolerance-development period. Voskuyl et al. (1986) suggested that the loss of DZP's anticonvulsant effect in rats that were regularly stimulated in the presence of the drug was attributable to an exacerbation of the seizures due to a "kindling-like effect" caused by the stimulation schedule used. However, the fact that the authors failed to note a similar kindling effect in control rats given the same number of stimulations raises the possibility that Voskuyl and his colleagues had unknowingly demonstrated contingent tolerance to DZP's anticonvulsant effects--that is, that the development of tolerance was contingent upon the rats receiving convulsive stimulation during periods of diazepam exposure.

Several clinical reports also support the idea that the occurrence of convulsions during periods of drug exposure might influence the development of tolerance to the anticonvulsant effects of antiepileptic drugs. Bruni and Albright (1983) reported that tolerance to VPA's anticonvulsant effect developed most rapidly in epileptics that demonstrated the highest incidence of seizures prior to the initiation of drug treatment.

Similarly, Meinardi et al. (1986) found that the development of tolerance to VPA's anticonvulsant effect emerged rapidly after the first instance in which the drug lost its efficacy. These data can be attributed to an exacerbation of the seizure disorder itself (as Meinardi et al., [1986] suggest), or to the fact that epileptics experiencing the most severe seizures are the least likely to respond to the effects of antiepileptic drugs (i.e., VPA was never truly effective in controlling the seizures in these patients). Alternatively, the same pattern of data would be expected if the development of tolerance to VPA's anticonvulsant effect is facilitated by the occurrence of convulsive activity during periods of drug exposure. The patients with the most severe seizures prior to drug treatment would be most likely to experience convulsive activity once drug treatment was initiated; this would accelerate the development of tolerance, so that it would appear to emerge rapidly once the first seizure was experienced.

2. Theories of Contingent Drug Tolerance: The Importance of Activity to the Development of Tolerance

Why should convulsive stimulation play such an important role in the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA? A more general question has been the focus of considerable attention in the area of contingent tolerance: Why does the activity of the drug recipient during periods of drug exposure play such an important role in the

development of tolerance to many drug effects? Three models have been proposed to account for the phenomenon of contingent tolerance: i) the reinforcement-density model; ii) the state-dependency model; and iii) the homeostatic-conditioning model of contingent tolerance. These models will be briefly reviewed in the next three subsections, and their utility as an explanation for contingent tolerance to anticonvulsant drug effects will be discussed.

i. The Reinforcement-Density Model of Contingent Tolerance

The reinforcement-density model of contingent tolerance (Corfield-Sumner & Stolerman, 1978; see also Demellweek & Goudie, 1983a,b; Schuster et al., 1966; Wolgin, 1989) is based upon the observation that tolerance to a drug's behavioral effects often develops only "when the initial effect of the drug causes a loss of reinforcement; when the drug has no effect on reinforcement or when it increases the frequency of reinforcement, no tolerance occurs." (Wolgin, 1989; p. 19). Based upon the principles of operant conditioning, the central idea in the reinforcement-density hypothesis of contingent tolerance is that tolerance to a drug's effects emerges as the drug recipient develops behavioral strategies that compensate for the drug effects that are responsible for the loss of reinforcement. The activity of the drug recipient during periods of drug exposure is important because it allows the drug recipient to interact with the reinforcement schedule that is in place; accordingly, only the

subjects in the drug-before-responding condition develop tolerance to the drug's effects on the criterion response because only these subjects experience the loss of reinforcement that results when the criterion response is performed while they are under the influence of the drug.

The reinforcement-density hypothesis provides a plausible explanation of those examples of contingent drug tolerance in which operant reinforcement principles are involved. For example, the reinforcement-density hypothesis provides a reasonable account of contingent tolerance to amphetamine's anorexigenic effects; the development of tolerance coincides with the acquisition of responses that compensate for the drug-induced stereotypy that interferes with the consummatory behavior (e.g., Salisbury & Wolgin, 1985; Wolgin, Thompson, & Oslan, 1987). Accordingly, this behavioral compensation can only occur if the rats are allowed to engage in consummatory behaviors while they are drugged (though see Wolgin, 1989, for alternative explanations for these data).

However, the generality of the reinforcement-density hypothesis is limited by at least five shortcomings. First, there have been few attempts to define the behavioral changes that compensate for the disruptive effects of the drug (Demellweek & Goudie, 1983b; Goudie, 1988; Wolgin, 1989; though see Wolgin & Salisbury, 1985; Wolgin et al., 1987); as Goudie has pointed out, "the empirical observation of contingent behavioral tolerance does not allow the conclusion that the mechanism by

which tolerance developed necessarily involved instrumental learning." (Goudie, 1988, p. 546). Second, Wolgin (1989) has noted instances in which contingent tolerance fails to develop to a drug effect that produces an obvious loss of reinforcement and other instances in which contingent tolerance develops to drug effects that increase the amount of reinforcement that a subject receives (see also Demellweek & Goudie, 1983b).

The remaining three weaknesses of the reinforcement-density model of contingent tolerance are particularly relevant to the present demonstrations of contingent tolerance to the anticonvulsant effects of CBZ, DZP, and VPA. The third shortcoming of the reinforcement-density hypothesis stems from its inability to account for instances of contingent tolerance that do not seem to involve a reinforcement process. For example, the reinforcement-density hypothesis cannot readily account for the development of tolerance to the anticonvulsant effects of the drugs; I am aware of no evidence that kindled seizures can serve as either a positive or a negative reinforcer and anticonvulsant drug effects would at least superficially appear to be beneficial to the drug recipient. The reinforcement-density hypothesis has similar difficulties accounting for contingent tolerance to the analgesic effects of ethanol (e.g., Jørgenson et al., 1985; 1986) or morphine (e.g., Advokat, 1989) in spinally transected rats, or to the effects of ethanol on the decay of posttetanic potentiation in the abdominal ganglia of Aplysia (e.g. Traynor et al., 1980).

The fourth shortcoming of the reinforcement-density hypothesis of contingent tolerance is its inability to account for the role that the drug dose and schedule of administration plays in the development of contingent and pharmacologic tolerance to a drug's effect. Pharmacologic tolerance develops regardless of whether or not the criterion response is performed during periods of drug exposure--and therefore in the absence of a loss of reinforcement.

The fifth, and final, shortcoming of the reinforcement-density hypothesis of contingent tolerance is that it cannot readily account for the effect that performance of the criterion response in the absence of drug exposure has on the dissipation of contingent tolerance to the anticonvulsant effects of ethanol (Mana & Pinel, 1987). Although the role of the criterion response in the dissipation of contingent tolerance has not been widely studied, it has been shown to be an important factor in the dissipation of contingent tolerance to amphetamine's anorexigenic effect (Poulos et al., 1981) and scopolamine's adipsic effect (Poulos & Hinson, 1984). A possible exception may be the report by LeBlanc et al., (1976), who found that contingent and pharmacologic tolerance to ethanol's effects on a moving-belt task dissipate at the same rate. However, these results may reflect the influence of behaviors common to both the criterion response (a moving belt task) and normal locomotion (e.g., ataxia; sedation) on the dissipation of tolerance, rather than a lack of effect of the criterion response.

ii. The State-Dependency Model of Contingent Tolerance

The term state-dependency refers to situations in which the efficient performance of a response is dependent upon a subject being tested in the same psychological state that existed when the response was acquired (Overton, 1966; 1984). According to the state-dependency hypothesis of tolerance (Chen, 1972; Cicero, 1980; Feldman & Quenzer, 1984; Wolgin, 1989), a response that was acquired by a subject in a drug-free state is poorly performed during periods of drug exposure because the drug-induced change in psychological state impairs the subject's ability to retrieve the information necessary to perform the task. The development of tolerance to this drug-induced impairment is presumed to reflect the acquisition of the response in the drugged state. Thus, instances of contingent tolerance are attributed to the fact that only the subjects in the drug-before group get the opportunity to acquire the criterion response while under the influence of the drug.

The utility of the state-dependency model as an explanation for contingent tolerance to the anticonvulsant effects of CBZ, DZP, and VPA is limited in at least four ways. First, it makes an explicit prediction that the convulsions of rats that are tolerant to the effects of an anticonvulsant drug should be impaired when the drug is withdrawn until the rats can reacquire the convulsive response in a drug-free state. Although the data in the present experiments do not address this issue, the results of earlier experiments from our lab provide no support for the

idea that drug withdrawal has any effect on the convulsions of kindled rats that are tolerant to anticonvulsant drug effects (Mana & Pinel, unpublished observations). Second, the state-dependency model of contingent tolerance cannot account for the development of pharmacologic tolerance to a drug's effects. Although this does not appear to be a serious limitation for a model of contingent drug tolerance, it is essential to an understanding of the phenomena of contingent and drug pharmacologic tolerance unless the two are completely independent entities. Third, the state-dependency model of contingent tolerance cannot account for the effect that undrugged performance of the criterion response has on the dissipation of tolerance to a drug's effects. And fourth, the role of cognitive processes in the state-dependency hypothesis of contingent tolerance limits its usefulness to reports of contingent tolerance in which a change in the psychological state of the subject might influence the retrieval of information required for the efficient performance of a task. For example, a state-dependency model is more capable of accounting for contingent tolerance to ethanol's effects on a subject's performance of a maze task than to its effects on more reflexive responses such as convulsions (e.g., Pinel et al., 1983), spinal reflexes (e.g., the tail-flick response; Jørgenson & Hole, 1985), or responses in reduced preparations (e.g., posttetanic potentiation in the isolated abdominal ganglion of Aplysia; Traynor et al., 1980).

iii. The Homeostatic-Conditioning Model of Contingent Tolerance

The homeostatic-conditioning model of drug tolerance was developed to describe the development and dissipation of contingent tolerance to the anorexigenic effect of amphetamine (Poulos et al., 1981) and to the adipsic effects of scopolamine (Poulos & Hinson, 1984). It represented an important advance in the study of behavioral influences on drug tolerance because it integrated the phenomena of context-specific tolerance and contingent tolerance into a single theory. According to Poulos and his associates, the development of contingent tolerance represents a homeostatic adaptation to a drug's effects on the criterion response. That is, the performance of the criterion response during periods of drug exposure is important to the development of tolerance because a hypothetical homeostatic regulator compares the subject's actual level of behavior to a set-point level appropriate to the organism's needs; "the level of consummatory behavior itself [rather than the need state; present author's note] constitutes a directly monitored biological system." (Poulos & Hinson, 1984, pp. 87). The subsequent manifestation of the homeostatic changes responsible for the development of tolerance is context-specific; that is, the manifestation of the tolerance is dependent upon the drug being administered in the same context that the subject previously experienced the drug's effects. Poulos and his colleagues argued that this contextual specificity is the product of Pavlovian conditioning. Finally, performance of the criterion

response in the absence of the drug leads to a dissipation of the physiological changes responsible for the development of tolerance as the drug recipient's homeostatic mechanisms reestablish a balance between the subject's needs and the consummatory response.

Although the synthesis of the areas of contingent and context-specific tolerance offered by the homeostatic-conditioning model is appealing, its utility as an explanation for contingent tolerance to anticonvulsant drug effects is limited in three ways. First, it is limited by the concepts of homeostasis and Pavlovian conditioning. Many instances of contingent tolerance do not involve an obvious homeostatic regulation of the criterion response involved or a dependence upon contextual stimuli; these would include instances of contingent tolerance to the anticonvulsant effects of drugs (e.g., Pinel et al., 1983; 1989), as well as contingent tolerance to the analgesic effects of ethanol (e.g., Jørgenson et al., 1985; 1986) or morphine (e.g., Advokat, 1989) in spinally transected rats and contingent tolerance to the effects of ethanol on the decay of posttetanic potentiation in the abdominal ganglia of Aplysia (e.g. Traynor et al., 1980). Second, the homeostatic-conditioning theory of contingent tolerance makes no prediction about the conditions that would lead to pharmacologic, as opposed to contingent, tolerance to a drug's effects. And third, the homeostatic-conditioning theory would predict that the criterion response should change following the cessation of drug

treatment until it can be homeostatically regulated to a level suitable for drug-free conditions. As mentioned earlier, drug withdrawal does not appear to affect the convulsions of kindled rats that have been rendered tolerant to the anticonvulsant effects of a drug.

Summary

The contingent tolerance phenomenon cannot be entirely accounted for by any one of the three existing theories (see also Wolgin, 1989; Goudie & Griffiths, 1986). One possible reason for this is the diversity of the phenomenon. Contingent tolerance has been demonstrated to the effects of a variety of pharmacologically disparate drugs: 1) psychostimulants (e.g., amphetamine, Carlton & Wolgin, 1971; Demellweek & Goudie, 1982; cocaine, Woolverton et al., 1979); 2) cannabinoids (e.g., delta-9-tetrahydrocannabinol, Manning, 1976); 3) sedative-hypnotics (e.g., Tizzano et al., 1986) and ethanol (e.g., Alkana et al., 1982; Chen, 1968; Pinel et al., 1983; 1989); 4) and opioids (e.g., morphine; Advokat, 1989; Kayan & Mitchell, 1969; Smith, 1979). The criterion response used to study contingent drug tolerance has also varied widely: 1) barpress responding (e.g., Branch, 1979; Woolverton et al., 1979); 2) drinking (Poulos & Hinson, 1984) and 3) feeding (e.g., Carlton & Wolgin, 1971); 4) nociception (e.g., Jørgenson & Hole, 1984); 5) posttetanic potentiation (Traynor et al., 1982); 6) thermoregulation (e.g., Alkana et al. 1982); 7) maze-running (Chen, 1968); 8) treadmill

running (LeBlanc et al., 1972); 9) mental rehearsal of a pursuit-rotor task (e.g., Sdao-Jarvie & Vogel-Sprott, 1986); and 10) convulsions (e.g., Pinel et al., 1983; 1989). The generality of the phenomenon and the magnitude of the effects generated by manipulating the occurrence of the criterion response during periods of drug exposure clearly indicate that the activity of the drug recipient during periods of drug exposure is a key factor in the development of many forms of drug tolerance; however, it is not clear whether any single theory of contingent tolerance could explain each instance of the phenomenon. In particular, the existing theories of contingent tolerance cannot easily account for the phenomenon of contingent tolerance to anticonvulsant drug effects or its relation to the phenomenon of pharmacologic tolerance to the same drug effects. Accordingly, an alternative conceptualization of contingent tolerance is presented in the next section.

3. An Activity-Dependent Analysis of Contingent and Pharmacologic Drug Tolerance

The development and dissipation of contingent tolerance can be summarized as follows: A change in neural activity during periods of drug exposure can influence the response of the neural system to the drug such that the drug subsequently has less of an effect on the activity. Conversely, when the same pattern or intensity of neural activity occurs in the absence of the drug, there is a functional change in these neural circuits such that

the effect of the drug on the activity is restored.

This axiom describing the development and dissipation of contingent tolerance to many drug effects bears a striking similarity to the more widely-recognized idea that concurrent activity of connected elements in a neural system has a key role in many forms of neural plasticity. Hebb (1949) is often acknowledged as the first to succinctly express the importance of this relationship, in what has become known as Hebb's Postulate:

> "When an axon of cell A is near enough to excite a cell B and repeatedly and persistently takes part in firing it, some growth or metabolic change takes place in one or both of the cells such that A's efficiency, as one of the cells firing B, is increased." (Hebb, 1949, pp. 62).

Stent's (1973) addendum to Hebb's Postulate captures the effect that asynchronous activity between the elements of a neural circuit has on the weakening of the connections between these elements:

> "When the presynaptic axon of cell A repeatedly and persistently fails to excite the postsynaptic cell B while cell B is firing under the influence of other presynaptic axons, some metabolic change takes place in one or both of the cells such that A's efficiency, as one of the cells firing B, is decreased." (Stent, 1973; pp. 997).

In the last decade, Hebb's Postulate has been expanded to

include synchrony between electrical and/or chemical changes in the postsynaptic neuron as a mechanism of Hebbian changes in synaptic transmission (e.g., Changeux & Heidmann, 1987). In the next three sections of the discussion, the role of concurrent activity in three different forms of neural plasticity--in the development of the neuromuscular junction, in the development of ocular dominance columns in the visual system, and in the development of long-term potentiation in the hippocampus--is reviewed. The purpose of this review is to illustrate the general importance of concurrent activity between the elements of a neural circuit to the plasticity of that circuit, and to provide a conceptual framework for an activity-dependent analysis of contingent and pharmacologic drug tolerance.

i. Concurrent Activity and the Neuromuscular Junction

The neuromuscular junction is one of the most widely studied synaptic connections in the nervous system. The outgrowth of the motor neuron from the spinal cord to the muscle appears to be a function of the neuron following a preferred substrate as well as the concentration gradient for some type of trophic factor secreted by the appropriate target tissue (e.g., Landmesser, 1980; see also Kandel, 1985a). However, once the motor neuron has reached the muscle tissue concurrent activity between the neuron and the muscle plays a critical role in the development of the appropriate synaptic connections. Prior to the muscle's ennervation, the nicotinic acetylcholine (n-ACh) receptor is

widely distributed over the muscle. Upon ennervation by the motor neuron, the distribution of this receptor (and hence the ennervation of the muscle by the motor neuron) becomes restricted to the motor end plate (see Kandel, 1985a). This change is not simply a result of the motor neuron ennervating the appropriate target zone but instead is a result of the interaction between the activity of the motor neuron and the muscle that it ennervates (e.g., Landmesser, 1980; Lømo & Rosenthal, 1972). When the motor neuron arrives at the muscle, the ACh that it releases stimulates the muscle to contract. These contractions lead to the degeneration of n-ACh receptors except in the area of the end plates; consequently, the muscle fibre is ennervated by a single motor neuron and only in the area of the motor end-plate.

The importance of the activity of both the muscle and the motor neuron to the proper development of the n-ACh synapse between them is illustrated by the fact that the formation of this connection can be prevented, or established connections weakened, by the application of tetrodotoxin (a drug that blocks the voltage-sensitive sodium channels necessary for nerve impulse activity) to the motor neuron (see Lømo & Rosenthal, 1972), or by the application of d-tubocurare (a n-ACh receptor antagonist that prevents the muscle from responding to the ACh released by the motor neuron) to the muscle (e.g., Landmesser, 1980). Although it is not clear that synchronous activity of the motor neuron and the muscle fibre is necessary for the establishment of the n-ACh synapse between the two (i.e., it is possible that the proper

synaptic connections would form if the motor neuron was simply close to a muscle fibre that was contracting because of exogenous stimulation), under normal circumstances the development of the n-ACh synapse between motor neurons and muscle fibres can be described as follows:

> When a motor neuron is near enough to activate a muscle fibre, and repeatedly and persistently takes part in its activation, some growth or metabolic change takes place in the muscle fibre that restricts the n-ACh receptor, and thus the cholinergic ennervation of the muscle, to the muscle's end plates. When the muscle is no longer stimulated by the neuron, the distribution of the n-ACh receptor spreads along the length of the muscle fibre until it is reinnervated.

ii. Concurrent Activity and the Development of Ocular Dominance Columns

Synaptic connections in the visual system undergo extensive reorganization during the course of normal development. A particularly striking example of this reorganization is the development of ocular dominance columns (e.g., Hubel, Weisel, & LeVay, 1977; see also Kandel, 1985b,c). The visual cortex is divided into six anatomically distinct layers; visual information travels via the retino-geniculo-striate path to neurons in layer IVc of the visual cortex, and from there to the remaining layers.

Early in development the cells in layer IVc respond to input from both eyes (at least in the cat; Singer, 1987); over time, the geniculo-striate connections change in such a way that cells in this layer of the visual cortex will respond to input from only one of the two eyes. This monocular preference alternates in a regular fashion across layer IVc; the neurons in these monocular patches project into the layers of visual cortex above and below them so that many of these neurons will also respond preferentially to input from one or the other of the two eyes (although there is considerably less preference in these layers because of the convergence of information from the different areas of layer IVc). These alternating columns of visual cortex, which respond preferentially to input from one or the other of the eyes, are referred to as ocular dominance columns.

The normal development of ocular dominance columns is believed to be the result of a Hebbian competition between the inputs from the two eyes for connections with cortical neurons in layer IVc of the striate cortex. The idea is that geniculo-striate synaptic connections are strengthened when converging input from one of the eyes to a given cortical neuron is sufficient to depolarize the cell and elicit an action potential; synapses that are not involved in the action potential are weakened. This hypothesis is supported by experiments showing that monocular, but not binocular, deprivation early in life alters the development of ocular dominance columns so that all cortical cells respond to input only from the nondeprived eye

(e.g., Weisel & Hubel, 1965). The lack of effect following binocular deprivation is attributed to the lack of competition between the eyes; neither eye can elicit an action potential from the cortical neurons so there is no change in synaptic strength. The importance of concurrence between the activity of the genicular input and the striate neurons to the development of ocular dominance columns was illustrated by Shaw and Cynader (1984), who found that chronic infusion of glutamate (an excitatory amino acid neurotransmitter that would increase the firing of cells in the area of the infusion which normally receive glutaminergic ennervation) into striate cortex during periods of monocular deprivation prevented the shift in ocular dominance that normally occurs. Presumably, the glutamate infusion prevented consistent concurrence between a given set of genicular inputs and the activity of the striate cortex neurons, thereby blocking the strengthening and/or weakening of geniculo-striate connections by a form of Hebbian competition.

This idea was recently extended by Bear, Kleinschmidt, Gu, and Singer (1990), who showed that chronic infusion of the n-methyl-d-aspartate (NMDA) antagonist APV (which blocks the NMDA subtype of glutamate receptor that appears to play a key role in many forms of activity-dependent neural plasticity) into striate cortex also prevents the ocular dominance shift that is normally produced by monocular deprivation. Similar results were reported by Reiter and Stryker (1988) following the microinjection of the GABA-A agonist muscimol into striate cortex during periods of

monocular deprivation. Clearly, normal postsynaptic activity plays a critical role in the development of ocular dominance columns; more interestingly, these data extend Stent's (1973) addendum to Hebb's Postulate; in some cases, synaptic weakening can occur when the postsynaptic element in a neural circuit is inactive and the presynaptic element is active, as well as when the postsynaptic neurons are active during periods when the presynaptic elements are not.

Finally, the contribution of normal presynaptic activity (i.e., the activity of genicular input from the eyes) to the development of ocular dominance columns was illustrated by Stryker and Harris (1986), who found that binocular blockade of retinal transmission in kittens by intravitreal injection of tetrodotoxin into the eyes (which would block the voltage-dependent sodium channels necessary for impulse conduction from the retina to the dorso-lateral geniculate) prevented the development of ocular dominance columns. Interestingly, Stryker and Harris (1986) found that intravitreal tetrodotoxin was more effective that dark-rearing or binocular suturing at preventing the development of ocular dominance columns; they argued that the spontaneous activity of retinal cells is sufficient to produce some segregation of genicular inputs in kittens that are deprived of normal visual experience.

In summary, the development of ocular dominance columns can be summarized by the following adaptation of Hebb's Postulate:

When genicular input to a striate neuron repeatedly and

persistently takes part in the firing of that neuron, some growth or metabolic change takes place to strengthen the common synaptic connections and weaken those from genicular inputs that did not participate in the activity of the striate neuron. When there is asynchrony between the activity of genicular inputs to striate cortex and the cortical neurons, there is a weakening of synaptic strength between these cells. Concurrent activity sufficient to depolarize the striate neurons in an given area of layer IVc is more likely to come from genicular input from one eye than the other; as a result, the connections from the less well-connected eye eventually weaken to the point that the striate cells in a given area of layer IVc respond preferentially to input from one eye only.

iii. Concurrent Activity and Long-term Potentiation

Perhaps the most well-studied example of the role that concurrent activity between the pre- and postsynaptic elements of a neural circuit can have in the plasticity of that circuit is the phenomenon of long-term potentiation (LTP; e.g., Bliss & Lømo, 1973; Bliss & Lynch, 1989; Gustafsson & Wigström, 1988). Long-term potentiation involves the prolonged enhancement of synaptic transmission following tetanic stimulation of the afferent neurons; although it has been most extensively studied in the circuitry of the hippocampus, LTP has been demonstrated

elsewhere in the central nervous system (e.g., Artola & Singer, 1987).

Several characteristics of LTP support a Hebbian model of neural plasticity. First, concurrent activity of both the pre- and postsynaptic neural elements is critical to the development of LTP. Hyperpolarization of the postsynaptic cell (so that it cannot respond to afferent input) blocks the development of LTP in spite of tetanic stimulation of the afferent neurons (e.g., Gustafsson, Wigström, Abraham, & Huang, 1987), and LTP can be induced by single afferent volleys (instead of tetanic stimulation of the afferent path) if each afferent stimulation is paired with a depolarizing current injection to the postsynaptic neuron (e.g., Wigström, Gustafsson, Huang & Abraham, 1986; see also Freidlander, Sayer, & Redman, 1990). The depolarizaton of the postsynaptic neuron alone, or the occurrence of single afferent volleys alone, are not sufficient to produce LTP (Wigström et al., 1986; see also Bliss & Lynch, 1988).

Support for Stent's (1973) addendum to Hebb's Postulate comes from studies by Steward and his colleagues (see Steward, White, Korol, & Levy, 1989, for a review) who have found that LTP established at connections between neurons in the entorhinal cortex and the contralateral dentate gyrus can be reversed if the neurons in the dentate gyrus subsequently receive tetanic stimulation from the ipsilateral entorhinal cortex in the absence of concurrent input from the cells in the contralateral entorhinal cortex. The authors noted, "Synapses that are

coactive with those inducing the modifiable state undergo potentiation; synapses that are silent during the modifiable state exhibit a long-term depression..." (Steward et al., 1989; pp. 139); an adequate summary of both Hebb's Postulate and Stent's addendum to that postulate.

iv. Summary

It is clear that concurrent activity between the pre- and postsynaptic elements in a neural circuit is an important factor in the plasticity at many different neural systems. Bear (1987) has suggested that "coincidence of activity may be the basic algorithm of activity-dependent changes in excitatory circuitry." (pp. 290); its role in the plasticity of neural circuitry as diverse as the hippocampus and the neuromuscular junction suggests that such a claim is not unrealistic (see also Changeux & Heidmann, 1987; Frank, 1987; Merzenich, 1987; Singer, 1987). The next section will extend the idea of activity-dependent neural plasticity to an analysis of contingent and pharmacologic tolerance to anticonvulsant drug effects.

v. The Role of Neural Activity in the Development of Functional Drug Tolerance: A Dissociation of Contingent and Pharmacologic Tolerance to DZP's Anticonvulsant Effect

Contingent tolerance to anticonvulsant drug effects can be distinguished from pharmacologic tolerance to the same drug effects in the following three ways:

1) Contingent tolerance requires less frequent administration, and/or smaller doses, of an anticonvulsant drug than pharmacologic tolerance (see Experiment 2 and Experiment 3, present thesis; Mana, Lê, Kalant, & Pinel, in preparation; see also Jørgenson et al., (1986), and Lê et al., (1986), for similar evidence from other types of contingent tolerance).
2) The development of contingent tolerance to anticonvulsant drug effects requires that convulsive stimulation is administered during periods of drug exposure; pharmacologic tolerance does not (Mana, Lê, et al., in preparation; Experiment 2 and Experiment 3, present thesis).
3) The dissipation of contingent tolerance to anticonvulsant drug effects is slower than that of pharmacologic tolerance (Experiment 4, present thesis) and may be influenced by the administration of convulsive stimulation in the absence of the drug (Mana & Pinel, 1987; see also Poulos et al., 1981; 1984; for similar evidence from other types of contingent tolerance).

In the text to follow, it is argued that these differences between contingent and pharmacologic tolerance can be accounted for by an analysis of functional drug tolerance that is based upon the level or pattern of neural activity occurring during periods of drug exposure.

The key assumption of this activity-dependent model of

contingent and pharmacologic tolerance is that the expression of a drug's effect is a function not just of factors associated with drug exposure (e.g., dose; schedule of administration), but also of the activity of the nervous system during periods of drug exposure. A corollary to this assumption is that the development of tolerance (T) will also be a function of both pharmacologic factors (P) and the activity of the neural circuits (N) during the periods of drug exposure--recall that functional drug tolerance develops not to drug exposure per se, but to the drug's effects on neural function (e.g., Kalant, 1985; Kalant et al., 1971; see also Pinel et al., in press). That is, the relationship between pharmacologic factors and neural activity to the development of drug tolerance can be represented by the following expression: T = P X N.

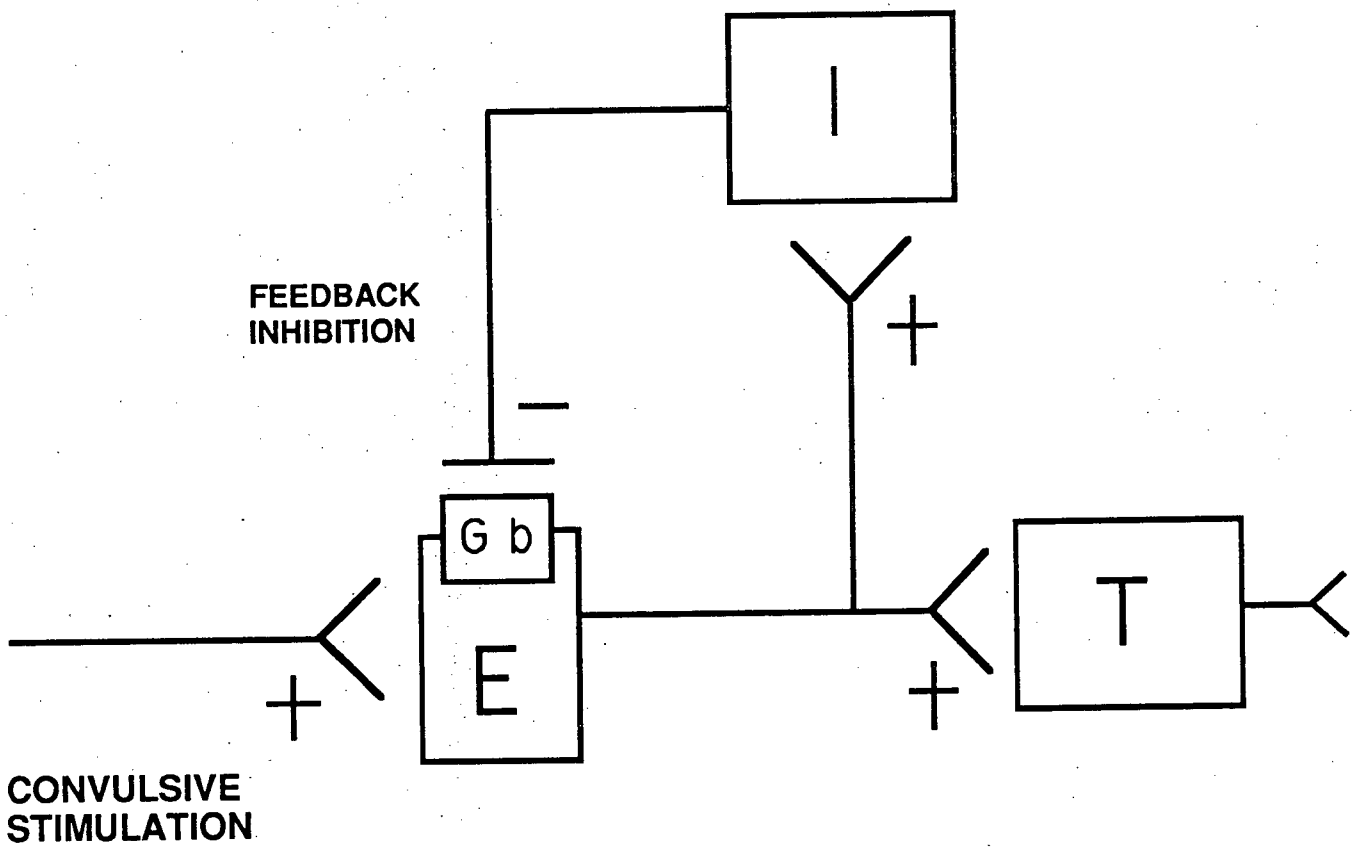
When an anticonvulsant drug is administered chronically, or at relatively high doses, the activity of the nervous system is less important to the development of tolerance to the drug's effects because the pharmacologic stimuli are of sufficient magnitude or duration that they disrupt the basal activity of the CNS to a degree sufficient to elicit the appropriate changes. Thus, pharmacologic tolerance can be represented by a slight change in the expression used to describe the development of tolerance; specifically: T = P X n (where the case of the letters in the right part of the expression denotes the relative contribution of the pharmacologic and activity-related factors to the development of tolerance). It must be stressed that a basal

amount of neural activity is assumed to be necessary for the drug effects to express themselves and functional tolerance to develop, even under optimal pharmacologic conditions; if the activity of the appropriate neural circuits could be eliminated during the periods of drug exposure then tolerance would not develop, regardless of the amount of pharmacologic stimulation that was available.

In contrast, the activity of the nervous system is more important to the development of tolerance when an anticonvulsant drug is given on a subchronic basis because the limited pharmacologic stimulation does not disrupt the basal activity of the nervous system enough to elicit the physiological changes responsible for the development of tolerance. In this situation, the development of tolerance to a drug's anticonvulsant effect is contingent upon convulsive stimulation affecting the pattern or intensity of neural firing during each period of drug exposure, so that the drug's anticonvulsant effect can be maximally expressed; in terms of the expression used earlier, contingent tolerance can be represented as: $T = p \times N$.

The application of these ideas to the development of contingent and pharmacologic tolerance to DZP's anticonvulsant effect is presented by the model neural circuit in Figure 15. In this simple system, there a population of excitatory neurons, designated E, that receive convulsive stimulation (either directly or from inputs from the primary site of seizure activity). Collateral axons from these

Figure 15. Model system illustrating several possible sites of physiologic adaptation that could serve as the physiologic basis for the activity-dependent distinction between pharmacologic tolerance and contingent tolerance to DZP's anticonvulsant effect. Pharmacologic tolerance may be due to a decrease in the sensitivity of the GABA-A receptors associated with the GABA/benzodiazepine complex (Gb) found on E-type excitatory neurons. Contingent tolerance may be due to a more widespread subsensitivity at these GABA-A receptors, to a more permanent expression of these changes, or to other changes in the GABA/benzodiazepine complex. Alternatively, it may be due to a change distant from the GABA/benzodiazepine complex--for example, in the connections between the excitatory neurons and their target neurons. See text for further details.


I
FEEDBACK
INHIBITION
G b
E
T
CONVULSIVE
STIMULATION

neurons synapse on inhibitory GABA-containing neurons, designated I. These inhibitory interneurons project back to synapse upon the GABA-A/benzodiazepine receptor complex, designated Gb, that are located on the soma and dendrites of the excitatory neurons. Thus, there is an inhibitory feedback loop that reduces the excitability of the excitatory neurons that is facilitated when DZP is present. The excitatory neurons also propogate the seizure activity to other structures in the brain, designated T or target neurons.

Even in this simple circuit, there are a number of different ways that an activity-dependent dissociation between contingent and pharmacologic tolerance to DZP's anticonvulsant effect could be expressed. For example, one possible mechanism is is based on the assumption that the sensitivity of the GABA-A receptor is a function of the concurrence of the occupation of the GABA-A receptor and the benzodiazepine receptor by their respective agonist ligands. Specifically, there is a decrease in the sensitivity of the GABA-A receptor when the GABA-A receptor and the benzodiazepine receptor are repeatedly occupied at the same time. Under basal conditions the concurrent occupation of both the GABA-A receptor and the benzodiazepine receptor is relatively infrequent because the basal activity of the circuit, and therefore the basal release of GABA, is low. Accordingly, DZP must be administered on a chronic basis for GABAergic subsensitivity to develop. When convulsive stimulation is administered, there is an increase in the release of GABA due to

an increase in the activation of the circuit; consequently, DZP can be administered less frequently and still elicit a significant decrease in GABAergic subsensitivity. The robustness of contingent tolerance to DZP's anticonvulsant effect could be due to a decrease in the sensitivity of a greater number of GABA-A receptors because convulsive stimulation elicits an increase in GABA release in the presence of DZP. Alternatively, there could be a more permanent change elicited at the GABA-A receptor when convulsive stimulation produces an increase in GABA release in the presence of DZP--perhaps because the occupation of both the GABA-A receptor and the benzodiazepine binding site during a convulsive stimulation results in a prolonged influx of chloride ions (because of the DZP-facilitated effect of GABA at the chloride channel associated with the GABA/benzodiazepine receptor complex), or because the allosteric modulation of the GABA receptor by DZP is concurrent with a tremendous change in membrane potential (due to the convulsive stimulation). This latter possibility could be extended to account for the possible role of convulsive stimulation in the dissipation of contingent tolerance as well--perhaps there is a return in the sensitivity of the GABA-A receptor and a dissipation of tolerance to DZP's anticonvulsant effect when there is a prolonged membrane depolarization (produced by convulsive stimulation) while the GABA receptor is unoccupied, or at least not occupied at the same time as the benzodiazepine binding site.

Although relatively simple, the proposed model accounts for

an activity-dependent dissociation of pharmacologic and contingent tolerance to DZP's anticonvulsant effect, both in terms of the development and the dissipation of these two forms of tolerance. However, it is possible--and likely--that a completely different type of physiological change could be responsible at least in part for the development and expression of contingent tolerance to DZP's anticonvulsant effect. For example, contingent tolerance to DZP's anticonvulsant effect could be due to an activity-dependent change in the synaptic connections between the excitatory neurons and their targets. A noteworthy feature of this possible mechanism of contingent tolerance to DZP's anticonvulsant effect is its emphasis on the idea that the physiologic changes that underlie such tolerance do not have to occur at the GABA/benzodiazepine complex; the fact that there is a binding site for the benzodiazepines in the CNS does not imply that changes in the efficacy of these drugs has to occur at this site. In particular, if this type of change is responsible for the development of contingent tolerance to DZP's anticonvulsant effect then such tolerance would not be affected by the administration of RO 15-1788 because the GABA/benzodiazepine receptor complex is not involved.

In summary, in an activity-dependent model of pharmacologic and contingent tolerance to anticonvulsant drug effects the occurrence of convulsive stimulation during periods of drug exposure can influence the development of tolerance in two ways. First, concurrence between convulsive stimulation and drug

exposure may facilitate the development of tolerance by facilitating the expression of the drug's anticonvulsant effect. This is especially important when a low, intermittent treatment dose is administered; this is recognized as contingent tolerance. Second, convulsive stimulation during periods of drug exposure may elicit a more robust set of, or even a different set of, physiologic adaptations to the drug's anticonvulsant effect; these are expressed as contingent tolerance.

Caveats and Final Comments

The activity-dependent dissociation between pharmacologic and contingent tolerance to DZP's anticonvulsant effect that was presented in the preceding section could be generalized to accommodate many of the various instances of contingent and pharmacologic tolerance that have been reported. For example, the idea of an activity-dependent change in synaptic transmission as a basis for drug tolerance could provide a useful starting point for examining the neural basis of instances of contingent tolerance involving relatively simple neural circuits (e.g., spinal reflexes such as the tail-flick response; reduced preparations such as the abdominal ganglia of Aplysia). It is less likely that such an approach could provide an immediate insight into the mechanism responsible for contingent tolerance involving more cognitively-mediated criterion responses (e.g., maze strategies; mental rehearsal of a task). As Kalant (1985) has pointed out, such examples of drug tolerance may prove to be

as difficult to understand as the phenomenon of learning itself--and although Hebbian modification of synaptic efficacy is widely regarded as a basic feature of the neural plasticity that underlies many forms of learning (e.g., Bear, 1987; Changeux & Heidmann, 1987), our understanding of the neural basis of learning is far from complete.

The hypothetical example of activity-dependent tolerance to DZP's anticonvulsant effect that was presented in the preceding section is pure speculation; to my knowledge, there is no evidence to support the idea of an activity-dependent modulation of the GABA-benzodiazepine receptor complex, or a role for some form of Hebbian modulation of either excitatory or inhibitory synapses, in the development of tolerance to the anticonvulsant effects of DZP or any other drug. Accordingly, the proposed mechanisms should be considered only as heuristic devices useful for conceptualizing the types of changes that could account for the phenomenological differences between pharmacologic and contingent drug--and perhaps as the basis for future studies into the physiological basis of tolerance to DZP's anticonvulsant effect.

3. Contingent and Pharmacologic Tolerance to DZP's Anticonvulsant Effect: Common or Independent Physiological Bases?

The first researchers to demonstrate the phenomenon of contingent tolerance frequently argued that it was not

explainable in terms of the physiological changes believed to underlie pharmacologic tolerance. Schuster et al. (1966) argued, "Clearly, the common physiological mechanisms responsible for drug tolerance cannot be appealed to as an explanation" (of contingent tolerance to amphetamine's effects on an operant task; p. 177); similarly, Chen (1968) believed that "In order to explain this type of tolerance effect, physico-chemical mechanisms are not sufficient..." (p. 439). However, this view has been impossible to support empirically; "The problem therefore remains whether the tolerance produced under these differing conditions is of different types, or is of a single type to which the additive effects of separate stimuli have contributed." (Kalant, 1989).

The results of the final two experiments in the present thesis reflected marked differences in both the spontaneous and the RO 15-1788-induced reduction of contingent tolerance and pharmacologic tolerance to DZP's anticonvulsant effect. In Experiment 4, pharmacologic tolerance to DZP's anticonvulsant effect dissipated over the 16-day retention interval; in contrast, there was no evidence of a decline in contingent tolerance to DZP's anticonvulsant effect over the same time period. The time course for the dissipation of pharmacologic tolerance to DZP's anticonvulsant effect was similar to that reported earlier by Rosenberg's group (e.g., Rosenberg et al., 1985; Rosenberg et al., 1986; Rosenberg et al., 1989) and by Gonsalves and Gallager (1987). Both of these groups have

suggested that pharmacologic tolerance to DZP's anticonvulsant effect is the result of a decrease in the sensitivity of the GABA-A receptor associated with the benzodiazepine binding site (e.g., Gallager et al., 1984; 1985; Gonsalves & Gallager, 1987; 1988; Rosenberg et al., 1985; Teitz & Rosenberg, 1988), which reduces the inhibitory effect of GABAergic transmission as well as the GABAergic facilitation of DZP binding that normally occurs at the GABA/benzodiazepine receptor complex. Accordingly, the difference in the rate of dissipation between pharmacologic and contingent tolerance to DZP's anticonvulsant effects on kindled seizures in Experiment 4 suggests that contingent tolerance to DZP's anticonvulsant effect is not attributable to the same decrease in GABAergic sensitivity.

The results of Experiment 5 support this hypothesis. In Experiment 5, a single injection of RO 15-1788 produced a significant attenuation in the expression of pharmacologic tolerance, but not contingent tolerance, to DZP's anticonvulsant effect. The reduction of pharmacologic tolerance to DZP's anticonvulsant effect by a single injection of RO 15-1788 extends earlier work by Gallager and Gonsalves (1988; see also Gonsalves & Gallager, 1985), and supports the idea that pharmacologic tolerance, but not contingent tolerance, to DZP's anticonvulsant effect is attributable to a decrease in the sensitivity of GABA-A receptors associated with the benzodiazepine receptor. The mechanism by which RO 15-1788 reverses the decrease in GABAergic sensitivity is unknown; however, Gonsalves & Gallager (1985;

1988; see Gallager, Rauch, & Malcolm, 1984) have suggested that the high-affinity, low-sensitivity state of the GABA-A receptor that is produced by chronic DZP treatment is allosterically reversed when the benzodiazepine receptor is occupied by an antagonist such as RO 15-1788.

The fact that RO 15-1788 had no effect on contingent tolerance to DZP's anticonvulsant effect supports the idea that GABAergic subsensitivity does not play a role in the expression of this form of tolerance. However, as noted in the discussion for Experiment 5 and in the preceding section of the General Discussion, the relative insensitivity of contingent tolerance to DZP's anticonvulsant effect to pharmacologic reversal by RO 15-1788 could also be due to an enhancement of an allosteric decrease in the sensitivity of the GABA-A receptor is presumed to undergo during the development of pharmacologic tolerance to DZP's anticonvulsant effect--such an enhancement could also be responsible for the fact that contingent tolerance to DZP's anticonvulsant effect did not dissipate over the 16-day retention interval used in Experiment 4, or following an injection of RO 15-1788. Thus, both pharmacologic tolerance and contingent tolerance to DZP's anticonvulsant effect could be due to a change in the sensitivity of GABA-A receptors, with contingent tolerance representing nothing more than a more extreme manifestation of this change. A resolution to this problem is beyond the scope of this thesis; however, the marked differences in spontaneous and pharmacologic dissipation of contingent and pharmacologic

tolerance to DZP's anticonvulsant effect suggest that this phenomenon could provide fertile ground for further analysis of the physiological bases of these phenomenologically distinct forms of tolerance. At the very least, the phenomenon of contingent tolerance may provide a more stable and easily detected set of physiological changes with which to study the phenomenon of drug tolerance at a more molecular level.

X. IMPLICATIONS.

i. Clinical Implications

The data from the five experiments contained in the present thesis are clinically relevant in at least two ways. First, the data from Experiment 2 clearly indicate that the occurrence of convulsive stimulation during periods of drug exposure can influence the development of tolerance to the anticonvulsant effect of antiepileptic drugs. These data are similar to those reported earlier by Killam et al. (1973), who found that tolerance to DZP's anticonvulsant effects developed faster in P. papio when the treatment dose of DZP was low enough that the baboons could experience infrequent convulsions. Killam et al. (1973) noted that the development of tolerance could be blocked by the administration of a high treatment dose of DZP; similar data have recently been noted by Kim and Pinel (unpublished observations), who found that the development of tolerance to pentobarbital's anticonvulsant effect could be retarded if a very high treatment dose was administered. Considered together, these

data support the idea that the development of tolerance to anticonvulsant drug effects could be reduced if the treatment dose used from the outset of treatment is high enough to effectively suppress all seizure activity and not just the convulsions produced by such activity (see also Koella & Meinardi, 1986b).

There are two obvious problems with such a treatment strategy. First, the use of a high treatment dose could reduce the likelihood of contingent tolerance developing to a drug's anticonvulsant effect at the same time that it promotes the development of pharmacologic tolerance to the same effects. This problem will be discussed further in the next paragraph. The second problem with such a strategy is that undesirable side-effects are also more likely with an increase in drug dose; accordingly, the treatment dose would have to compromise between one high enough to prevent the development of tolerance and one low enough to maintain an acceptable therapeutic index for the drug.

The second clinical implication of the present data concerns the marked reduction of tolerance to DZP's anticonvulsant effect that was produced by a single injection of the benzodiazepine antagonist RO 15-1788. The development of tolerance to the anticonvulsant effects of the benzodiazepines has been widely acknowledged as the major reason why this class of antiepileptic drug is not widely employed in the treatment of the epilepsies (see Engel, 1989; Haigh & Feely, 1988; Robertson, 1986), and

considerable attention has been devoted to the development of new benzodiazepine ligands that are less prone to the development of tolerance (e.g., Haigh & Feely, 1988). The results of Experiment 5 suggest that periodic administration of RO 15-1788 (coadministered with a nonbenzodiazepine antiepileptic to control seizures while the benzodiazepine receptor is occupied by the RO compound) might prevent the development of pharmacologic tolerance to the anticonvulsant effects of existing benzodiazepine antiepileptic drugs. Although RO 15-1788 was ineffective in reversing contingent tolerance to DZP's anticonvulsant effect, this problem might be reduced if a high treatment dose of DZP was used (to reduce the likelihood of contingent tolerance) in conjunction with periodic administration of RO 15-1788 and a nonbenzodiazepine anticonvulsant (to reduce the development of pharmacologic tolerance).

ii. The Activity-Dependent Model of Drug Tolerance

Perhaps the most interesting feature of this thesis is the introduction of a model of activity-dependent drug tolerance. This model has several advantages over earlier models of contingent drug tolerance. First, it integrates the phenomenon of contingent tolerance into the larger literature of pharmacologic drug tolerance; the relationship between these two phenomenologically distinct processes has not been explicitly dealt with in earlier models of contingent tolerance. Second, an activity-dependent model of drug tolerance provides some specific predictions about the factors that determine which type of

tolerance will develop and why they are important, as well as some predictions about the types of physiological change that could underlie the development of pharmacologic or contingent tolerance. Finally, an activity-dependent model of drug tolerance integrates the study of pharmacologic and contingent drug tolerance into the much larger literature on activity-dependent change in the nervous system that has developed in other areas of the neurosciences. This type of integration is critical to a better understanding of the relationship between contingent and pharmacologic tolerance and the phenomenon of drug tolerance in general. As Kalant (1989) recently observed:

> "From the biologists point of view, a purely behavioral explanation of drug tolerance...is a description, rather than an explanation, unless it attempts to link the behavioral influences with cellular mechanisms. Similarly, a cellular explanation is not an explanation if it does not take into account the very important influences that behavioral and environmental factors can exert upon the development of tolerance....Unless both approaches are employed in an integrated manner, it will be impossible to answer the question as to whether the various behavioral and pharmacological factors elicit the same type of tolerance or act through entirely different mechanisms." (pp. 572).

The purpose of this thesis was to contribute to such an integrative approach to the study of drug tolerance.

XI. References

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