WITHDRAWAL-PRODUCED INCREASE IN SUSCEPTIBILITY TO KINDLED SEIZURES FOLLOWING A SINGLE INJECTION OF ALCOHOL IN RATS

by

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ABSTRACT

It has been well-established in both humans and laboratory animals that the withdrawal of alcohol after a period of chronic exposure can have convulsive effects. In the present studies, however, such convulsive effects were detected in naive rats following the metabolism of a single ethanol injection. These effects were assessed by measuring the duration of kindled motor seizures (MSs) and electrographic after discharges (ADs) elicited by low-intensity amygdaloid stimulation at intervals before, during, and after the exposure. Kindled MSs are those which can be reliably elicited only after an organism has been stimulated periodically with an initially ineffective low-intensity current. Thus, before each experiment the subjects were kindled with the periodic amygdaloid stimulation until MSs and ADs of stereotypical duration and pattern were reliably elicited. The consistency of the kindled seizures provided a stable baseline against which to assess the convulsive effects of the withdrawal of ethanol.

In Experiment 1 kindled seizures were elicited at fixed intervals before and after a single intraperitoneal injection of ethanol. Changes in the AD and MS duration revealed a potent anticonvulsive effect in the 3 hr immediately following the injection, followed about 12 hr later by a convulsive effect lasting about 5 hr. In Experiment 2 this effect was replicated and directly related to changes in the level of blood ethanol. The anticonvulsive effect was related to the presence of the ethanol while the convulsive effects seemed to be triggered by its metabolism. In Experiments 3 and 4 a different experimental design was used to study the

effects of single intraperitoneal and intragastric injections, respectively. Since in these experiments only one seizure was elicited in each subject after the alcohol injection, the possibility that the increase in seizure duration was an artifact of the repeated testing procedure rather than a bona fide withdrawal-produced effect was ruled out.

Thus, the results confirmed previous demonstrations of convulsive effects following the metabolism of single injections of ethanol. This established the generality of the phenomenon to other species, modes of ethanol administration, and to different experimental designs; and the utility of the kindling paradigm for studying the time course of anti-convulsive and convulsive effects. The changes underlying the manifestation of severe convulsive effects obvious in humans after withdrawal from chronic ethanol exposure appears to develop with an individual's first exposure.

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INTRODUCTION

Ethanol in moderate to high doses has well-known depressant effects on various levels of nervous system functioning (cf. Himwich & Callison, 1972; Israel, 1970; Kalant, 1970). Ethanol inhibits the utilization of glucose in the brain (Roach, 1970), inhibits action potentials (Armstrong & Binstock, 1964), decreases in both frequency and amplitude spontaneous electroencephalographic (EEG) activity (Horsey & Akert, 1953), and reduces the amplitude of evoked potentials in various parts of the mammalian brain (Himwich & Callison, 1972). Ethanol has also been shown to have strong anticonvulsant properties (Allan & Swinyard, 1949; McQuarrie & Fingl, 1958). In apparent contradiction to these depressant effects is the well-established relation between the use of ethanol and the occurrence of seizures. One of the earliest available reports of such a relation was by a ship's surgeon (Trotter, 1804) who noted that some epileptic fits and facial tremors were sometimes associated with consumption of alcoholic beverages. A similar report of the relation between chronic alcohol consumption and seizures was published by Hubbard in 1881. Since these early reports, alcohol-related convulsions have been repeatedly described in both the clinical and experimental literature (cf. Bowman & Jellinek, 1942; Echeverria, 1881; Essig, 1972; Isbell, Fraser, Wikler, Belleville, & Eisenman, 1955; Victor, 1968).

The most obvious interpretation of these alcohol-related convulsions was that the presence of the alcohol triggered the convulsions. However, lack of experimental evidence showing that alcohol was a convulsant (cf. Berry, 1952) led investigators to consider the possibility that the

production of seizures was mediated by intermediate pathological effects of the alcohol. Pellagra (Langworth, 1931), vitamin B_6 deficiency (Lerner, De Carli, & Davidson, 1958), shifts in blood plasma volume (Nicholson & Taylor, 1940), magnesium deficiency (Klingman, Suter, Green, & Robinson, 1955), hepatic diseases (Froment, Masson, & Brun, 1939), cerebral edema (Cobb, 1932), and cerebral atrophy (Lafron, Pages, Pussouant, Labunge, Minvielle, & Cadilhac, 1956) have all at one time been considered as factors in the production of alcohol-related convulsions. Some authors believed that alcohol precipitates convulsions in only those already susceptible (Lennox, 1941) while others attributed the relation between alcohol consumption and convulsions to the presumed tendency of epileptics to consume large amounts of alcohol (Echeverria, 1881).

More recent clinical and experimental research has discounted these interpretations of alcohol-related convulsions by providing clear evidence that it is the sudden reduction in alcohol consumption following sustained intoxication which elicits the convulsions (Isbell et al., 1955; Kalinowsky, 1942; Victor & Adams, 1953; Victor & Brausch, 1967). Kalinowsky (1942), for example, in an early report emphasized the similarity between alcohol-related convulsions and those elicited when chronic exposure to barbiturates and sedatives is stopped. Among the more widely quoted studies providing evidence that alcohol withdrawal is responsible for triggering alcohol-related convulsions are the reports of Victor and his colleagues (Victor & Adams, 1953; Victor & Brausch, 1967) based on the carefully documented observations of alcoholics requiring hospital attention for seizures. Their major finding was that convulsions typically occurred between 6 and 48 hr after the cessation of drinking rather than during

periods of intoxication. Only a small percentage of patients had any evidence of spontaneous and recurrent convulsions or EEG epileptiform discharge, or a history of severe head trauma, thus the possibilities of latent epilepsy producing the seizures and of epilepsy giving rise to alcohol use were discounted. Victor and his coworkers found withdrawal seizures to be elicited only if the cessation of drinking followed a long period of relatively continuous alcohol consumption. Five days was the shortest period of exposure which they observed to culminate in withdrawal seizures.

The results of Victor and coworkers' clinical studies are similar to those of an experiment by Isbell et al. (1955). The subjects were healthy former morphine addicts who were maintained on nutritious diets and ethanol for periods ranging from 48 to 87 days. The subjects voluntarily consumed 388-489 ml of alcohol daily maintaining average blood ethanol levels between 200 and 300 mg per 100 ml of blood over the drinking period. When alcohol administration was discontinued, all six subjects completing the study became tremulous, hyper-reflexic, sweated profusely, lost weight, and experienced insomnia; two had convulsions, five developed hallucinations, and three became disoriented. Dysrhythmias in the form of spike-and-wave discharges were present in the three subjected to EEG analysis (Wikler, Pescor, Fraser, & Isbell, 1956). The four patients that withdrew from the study within 34 days displayed only minor withdrawal-produced symptoms.

PURPOSE

Thus, it is now well-established that alcohol-related convulsions are triggered by the withdrawal rather than the administration of alcohol. Clinical investigations and experiments on both human and animal subjects have consistently found that withdrawal-produced convulsions are not elicited until there have been many weeks of exposure (Deneau, Yanagita, & Seevers, 1969; Essig & Lam, 1968; Isbell et al., 1955). There is some evidence, however, which suggests that some withdrawal-produced convulsive effects may be observed following an organism's first brief exposure to ethanol. Goldstein (1972) and McQuarrie and Fingl (1958) found a small but detectable increase in the susceptibility to experimentally-elicited convulsions in naive mice a few hours following administration of a single dose of ethanol. The study of this indication of early withdrawal-produced convulsive effects may eventually provide valuable insights into the etiology of alcohol-related convulsions and of various disorders related to alcohol consumption. However, with the exception of the two aforementioned studies there is very little research on convulsive effects after single doses of ethanol. Thus, the purpose of the present experiments was to study these convulsive effects produced by exposing a naive organism to a single injection of ethanol.

In the present series of experiments the anti-convulsant and convulsant consequences of a rat's first exposure to ethanol were assessed by measuring the duration of kindled seizures at various intervals before and after the administration of the ethanol. Kindled seizures are those elicited in animals previously kindled with periodic low-intensity bipolar stimulation

of the brain (Goddard, McIntyre, & Leech, 1969). In the present studies, rats were kindled with periodic amygdaloid stimulation administered at an intensity which was initially too low to elicit motor seizures (MSs). Eventually, however, each stimulation elicited a full clonic MS and it was the changes in the MS and the after discharge (AD) durations which were used to measure the effects of a single alcohol exposure. The development of this sensitive technique of measuring the time course of convulsive and anticonvulsive effects was a secondary purpose of the present investigation.

LITERATURE REVIEW

Thus, in view of the two purposes, this series of experiments is relevant to two separate experimental phenomena; alcohol-withdrawal-produced convulsive effects and kindling. There are three aspects of alcohol-withdrawal-produced convulsive effects that are particularly pertinent. These are 1) studies of convulsions occurring spontaneously following withdrawal, 2) studies of increased susceptibility to experimentally-elicited seizures after alcohol withdrawal, and 3) studies of the effects of the duration of the alcohol exposure on the convulsive effects of withdrawal. The kindling literature relevant to the present paper deals specifically with the use of kindled seizures to assess changes in seizure susceptibility.

Alcohol-Withdrawal-Produced Convulsive Effects

With a few notable exceptions (Isbell et al., 1955; Mendelson, Stein, & McGuire, 1966) investigations into the physiological basis of alcohol withdrawal seizures have typically involved laboratory animals. The experimental control necessary to attack problems related to withdrawal convulsions is not usually possible in studies involving human subjects.

1. Spontaneous Withdrawal Effects

Withdrawal-produced convulsions have been difficult to elicit in experimental animals because they will not readily consume high levels of alcohol (Myers & Veale, 1972). Thus, the main problem has been to administer sufficient quantities of ethanol to maintain long periods of exposure. A number of methods of administering ethanol so that withdrawal

convulsions are eventually produced have been reported. These methods and their effects are summarized in Table 1.

Until 1968 there was very little direct evidence that alcohol withdrawal convulsions could be produced in animals. Indirect evidence consisted of observations of convulsions produced by barbiturate withdrawal in rats (Essig, 1966), dogs (Fraser & Isbell, 1954), and cats (Essig & Flanary, 1959). Essig and Lam (1968) provided the first published demonstration of alcohol withdrawal convulsions in animals. Ethanol was administered via an intragastric cannula several times a day to dogs maintained on a vitamin-enriched diet; the dogs were water deprived to encourage additional oral intake of ethanol. After 54 days of exposure the alcohol was withheld and five of eight subjects experienced convulsions between the 11th and 48th hr of abstinence.

The intragastric route of administering alcohol has been the most widely used method of inducing withdrawal convulsions. However, with the exception of one study on cats by Guerrero-Figueroa, Rye, Gallant, & Bishop (1970) in which permanent gastric fistulae were used, gavage has been the method of choice for administering the ethanol. The primary advantage of gavage is that it does not involve the surgical implantation of the cannulae; a tube is introduced into the stomach of the subject via the esophagus, the alcohol injected, and the tube removed. This technique has been effective in producing withdrawal effects in dogs (Ellis & Pick, 1972), monkeys (Ellis & Pick, 1970, 1971), and rats (Hunt, 1973; Majchrowicz, 1973; Mucha, Pinel, & Van Ot, 1975; Wallgren, Kosunen, & Ahtee, 1973).

Another method of producing alcohol withdrawal convulsions in laboratory animals that, like the intragastric techniques, permits strict

Table 1. Methods of administering ethanol for producing withdrawal convulsions in various species.

Investigator(s)	Species	Method of Ethanol Administration	Dose	Duration of Exposure	Epileptic Symptoms
Branchey, Rauscher & Kissen (1971)	rat	ethanol-rich diet in weight-restricted animals	4.3/ml/rat/day	21 days	clonic convulsions
Cannon, Baker, Berman, & Atkinson (1974)	rat	gavage	8-12g/kg/day	2 to 4 days	increased susceptibility to audiogenic seizures
Deneau, Yanagita, & Seevers (1969)	monkey	intravenous self-administration	8.6g/kg/day	about 5 months	convulsions, clonic-tonic convulsions
Ellis & Pick (1970, 1972)	monkey	nasogastric gavage	4-8g/kg/day	10 to 18 days	clonic-tonic convulsions
Ellis & Pick (1972)	dog	gavage	5.8-6.5g/kg/day	2 to 4 weeks	convulsions
Essig & Lam (1968, 1969)	dog	intragastric infusion	4-4.5ml/kg of 40% at 4 hr intervals	54 days	convulsions
Falk, Samson, & Winger (1972)	rat	schedule-induced polydipsia	13.1g/kg/day	3 months	increased susceptibility to audiogenic seizures
Freund (1969)	mouse	ethanol-rich diet in weight-restricted animals	.51-.53ml/day	4 days	generalized tonic-clonic convulsions
Freund (1971)	mouse	ethanol-rich diet in weight-restricted animals	.40ml/day	4 days	tonic-clonic convulsions and running fits
Freund (1971)	mouse	ethanol-rich diet in mice with 4°C housing	.40-.37ml/day	1 week	tonic-clonic convulsions and running fits
Goldstein (1972)	mouse	inhalation of ethanol vapour with pyrazole	vapour concentration of 11mg/litre	1-9 days	increased susceptibility to handling convulsions
Goldstein & Pal (1971)	mouse	inhalation of ethanol vapour with pyrazole	vapour concentration of 12mg/litre	4 days	spontaneous convulsions, increased susceptibility to handling convulsion
Guerrero-Figueroa, Eye, Gallant, & Bishop (1970)	cat	intragastric infusion	18-31ml/day of 40%	2-5 months	tonic-clonic convulsions
Hunt (1973)	rat	gavage	11-15g/kg/day	7 days	clonic-tonic convulsions, lower pentylenel-tetrazol threshold
Hunter, Boast, Walker, & Zornetzer (1974)	rat	ethanol-rich diet in weight-restricted animals	15.5g/kg/day	15 days	convulsions, EEG epileptiform activity
Isbell, Fraser, Wikler, Belleville, & Eisman (1955)	man, former morphine addicts	oral ingestion	388-489ml/day	48-87 days	convulsions, EEG epileptiform activity
Lieber & De Carli (1973)	rat	ethanol-rich diet	14-16g/kg/day	2-4 weeks	increased susceptibility to audiogenic seizures
Majchrowicz (1973)	rat	gavage	12-15g/kg/day	3-5 days	convulsions
McQuarrie & Fingl (1958)	mouse	gavage	5.4g/kg/day	14 days	decreased threshold to electroconvulsive seizures
Mucha, Pinel, & Van Oot (1975)	rat	gavage	8.5-10.4g/kg/day	7-30 days	convulsions, increased susceptibility to audiogenic seizures, EEG epileptiform activity
Ortiz, Griffiths, & Littleton (1973)	mouse	inhalation of ethanol vapour	vapour concentration of 8-25mg/litre	10 days	increased susceptibility to handling convulsions
Ratcliffe (1972)	rat	increasing concentrations of ethanol as only available fluid	(no data)	5-7 weeks	increased susceptibility to audiogenic seizures
Roach, Khan, Coffman, Pennington, & Davis (1973)	rat	inhalation of ethanol vapour	vapour concentration of 15-30mg/litre	7 days	increased susceptibility to handling convulsions
Walker & Zornetzer (1974)	mouse	ethanol-rich diet in weight-restricted animals	35.5mg/kg/day	6 days	convulsions, EEG epileptiform activity
Wallgren, Rosunen, & Ahtee (1973)	rat	gavage	6-12g/kg/day	18-21 days	convulsions

Experimental control of ethanol administration involves housing animals in an atmosphere of ethanol vapour. Alcohol was first administered to experimental animals via this route by Goldstein and Pal (1971) who administered the vapour to mice injected with pyrazole, an inhibitor of alcohol dehydrogenase activity, which insured higher, more stable levels of blood ethanol. Withdrawal-produced convulsions were observed in some mice after as little as three days of exposure.

Restricting consumption of weight-reduced animals to ethanol-rich diets is another technique commonly employed in the study of withdrawal-produced convulsions. Freund (1969, 1971) was the first to use this in the study of withdrawal convulsions. Mice were reduced to 65% or their normal weight and were then given diets restricted to Metrecal and alcohol mixed so that 35% of the calories were supplied by the ethanol. Convulsions were produced when the alcohol in the diet was replaced with sucrose after four days of exposure. Walker and Zornetzer (1974) produced convulsions after exposing mice restricted to 85 to 90% of their normal weight to the ethanol diet for six days. In addition, EEG examination during withdrawal revealed widespread epileptiform activity in forebrain structures.

This technique has also been used to study withdrawal-produced convulsions in rats. Branchey, Rauscher, and Kissen (1971) found that clonic convulsions were produced in rats deprived to 66% of their normal weights when the alcohol-Metrecal solution was withdrawn after 21 days of exposure. Hunter, Boast, Walker, and Zornetzer (1973) found 15 days sufficient to produce convulsions and EEG epileptiform activity in rats reduced to 75% of their original weight.

Considerable criticism has been levelled at the use of weight-reduced animals to study withdrawal convulsions (Littleton, Griffiths, & Ortiz, 1974; Mello, 1973; Ogata, Ogato, Mendelson, & Mello, 1972; Wallgren et al., 1973). Ogata et al., (1972) concluded that severe nutritional deficits contribute significantly to withdrawal effects. Of two groups of mice consuming the same quantity of ethanol, only animals in the nutritionally-deprived group developed withdrawal symptoms. The fact that alcohol metabolism in fasted animals is decreased by as much as 50% (Owens & Marshall, 1955) may have been the basis for this effect.

Thus, techniques employing ethanol-liquid diets but not requiring weight reduction have been developed for the study of alcohol withdrawal seizures. Freund (1971) reported that convulsive symptoms could be seen in normal-weight rats if they were fed diets with 18% of the calories constituted by ethanol and housed at about 4°C for one week. Similarly, Pieper, Skeen, McClure, and Bourne (1972) observed tonic-clonic withdrawal convulsions in young, healthy chimpanzees after six to 10 months of exposure to a liquid diet in which 45% of calories were comprised of ethanol.

Intravenous self-administration by lever pressing was first shown to be useful for studying withdrawal-produced effects in a morphine experiment by Weeks and Collins (1968). A year later Deneau et al., (1969) provided the first published report of animals self-administering ethanol. Monkeys chronically self-injected up to 8.6 g/kg per day and developed convulsions when the ethanol administration was stopped.

2. Increases in Susceptibility to Experimentally-Elicited Convulsions

The detection of withdrawal-produced convulsive effects by the presence or absence of spontaneous withdrawal convulsions has two major

limitations. First, there is evidence of convulsive effects in subjects not displaying spontaneous withdrawal seizures. Wikler et al., (1956), for example, found that alcohol withdrawal triggered epileptiform electrographic abnormalities in subjects not displaying overt convulsions. Motor convulsions are generally all-or-none events and do not provide the investigator with a method of detecting mild convulsive effects after alcohol withdrawal. Second, in order to detect the presence of withdrawal convulsions, subjects must be observed continuously during the withdrawal period. Victor and Adams (1953) indicated that those patients experiencing withdrawal convulsions rarely have more than one or two during their entire abstinence period.

An alternative means of identifying the convulsive effects of alcohol withdrawal is to assess increases in the susceptibility to seizures triggered by convulsive agents during the withdrawal period. The rationale for this method is based on the well-established observation that epileptic effects are additive (cf. Ajmone-Marson & Ralston, 1957). Thus, an epileptic agent administered at doses which do not themselves elicit convulsions will intensify those produced by another convulsive agent. This additive feature has been widely used to detect epileptic developments in patients not displaying spontaneous convulsions or electrographic seizure activity. For example, a small dose of pentylenetetrazol, ineffective in healthy subjects, will frequently trigger seizures in suspected epileptics (cf. Ajmone-Marson & Ralston, 1957).

There is a direct relation between the presence of alcohol-withdrawal-produced spontaneous seizures and changes in susceptibility to convulsive agents. This relation was clearly evident in a study by Victor and

Brausch (1967) in which sensitivity to photic stimulation was assessed in patients undergoing alcohol withdrawal. The photic stimulation consisted of flashes of light delivered at frequencies from 4 to 24 flashes per sec. This photic stimulation rarely provoked epileptic responses in normal individuals or in diagnosed epileptics but patients undergoing withdrawal of alcohol developed convulsive responses in about 50% of the cases. Furthermore, the periods in which susceptibility to light-elicited effects was the greatest were the same periods in which spontaneous convulsions were most prevalent. Similarly, Goldstein (1972) reported a high positive correlation between spontaneous and handling-elicited convulsions in mice following the cessation of exposure to ethanol vapour. Ratcliffe (1972) observed withdrawal-produced increases in susceptibility to audiogenic seizures in 40 to 80% of the rats restricted to ethanol-rich diets for five to seven weeks, respectively. Only three of 300 similarly exposed rats were observed to have spontaneous convulsions. Thus, it seems that increases in the susceptibility to convulsive agents after alcohol withdrawal provide a more reliable and sensitive measure of convulsive effects of alcohol withdrawal than the increases in the incidence of spontaneous seizures.

Methods employed to produce alcohol withdrawal convulsions in experimental animals have increased the susceptibility to a variety of convulsive agents (see Table 1). In rats, withdrawal following chronic alcohol exposure by gavage increases susceptibility to audiogenic seizures (Cannon, Baker, Berman, & Atkinson, 1974; Mucha et al., 1975), susceptibility to pentylenetetrazol-produced convulsions (Hunt, 1973; McQuarrie & Fingl, 1958) and susceptibility to convulsions elicited by handling (Cannon et al., 1974). The withdrawal following presentation of

ethanol-liquid diets to weight-reduced rats (Hunter et al., 1974) and mice (Freund & Walker, 1971) and non-weight-reduced rats (Lieber & De Carli, 1973) has increased the susceptibility to audiogenic seizures. Chronic alcohol consumption produced by schedule-induced polydipsia (cf. Lester, 1961; Holman & Myers, 1968; Ogata et al., 1972) has been reported to result in a withdrawal-produced increase in the susceptibility to audiogenic seizures (Falk, Samson, & Winger, 1972). In many of these experiments the increased susceptibility to various convulsive agents occurred in the absence of spontaneous convulsive effects.

3. Duration of Alcohol Exposure and Convulsive Effects of Withdrawal

Isbell et al. (1955) clearly showed that the severity of withdrawal reactions is an increasing function of the amount of previous ethanol exposure. Subjects that were exposed to high levels of alcohol for 48 to 87 days experienced convulsions and epileptiform EEG activity, while subjects that were exposed for fewer than 34 days did not. This relation has also been demonstrated in a variety of infra-human species. Mucha et al. (1975), for example, administered ethanol to rats by gavage for 7, 15, or 30 days and found the incidence of spontaneous convulsive symptoms to be an increasing, negatively accelerated function of the duration of ethanol exposure. Using similar methods, Majchrowicz (1973) found a high incidence of withdrawal seizures after three to five days but not after one or two days of exposure.

The same relation has been observed when the convulsive effects of the alcohol withdrawal are gauged in terms of the susceptibility to elicited seizures rather than in terms of the incidence of spontaneous

seizures. Lieber and De Carli (1973) found that 10 to 14 but not 5 to 7 days of forced exposure to a diet in which 36% of the calories were comprised of ethanol increased susceptibility to audiogenic seizures. Similarly, Goldstein (1972) found that the incidence of handling-elicited withdrawal convulsions increased with the duration of intoxication produced by ethanol vapour.

Two series of experiments demonstrating increased seizure susceptibility following brief exposures to ethanol are particularly relevant to the present studies. The first experiment was carried out by McQuarrie and Fingl (1958). They found that following the administration of 4.0 g/kg of alcohol to mice via gavage there was a brief period of increased susceptibility to pentylenetetrazol-induced seizures. Eight and 12 hr after ethanol administration threshold was decreased, while tests at 24 hr failed to reveal any difference between the thresholds of ethanol and control animals. McQuarrie and Fingl (1958) claim to have observed a similar pattern of change in electroshock thresholds in a similar experiment, but did not publish the results.

The second incidence of withdrawal-produced convulsive effects after a single injection of ethanol was reported by Goldstein (1972). Mice injected with 5.0 g/kg of ethanol i.p. and 1.0 mmole/kg of pyrazole displayed mild increases in susceptibility to handling-elicited convulsions 10 to 11 hr after the injection. The peak effect occurred at about 7 hr when no pyrazole was injected. A lower dose of alcohol, 2.0 g/kg, administered without pyrazole increased the susceptibility to handling convulsions 3 hr after the injection. The only systematic determination of the blood alcohol levels was made on the animals receiving pyrazole. Ethanol elimination curves indicated that the peak withdrawal scores occurred while

some ethanol was still present in the blood.

Similar studies have not been performed with human subjects. Human subjects employed in alcohol experiments typically have a long history of drinking. However, research has indicated that the duration of ethanol exposure required to produce withdrawal convulsions in these subjects is considerably less than once thought. Victor (1968) and Berry (1952) have reported that only one evening of heavy drinking is enough to increase the severity and incidence of seizures in some epileptics. Similarly, Nagy, Zsadanyi, Nagy, and Zsigmond (1973) found that following the metabolism of only one or two small quantities of an alcoholic beverage there was photically- or hyperventilation-induced epileptic activity in the EEG of some clinically healthy subjects. In those few patients who had spike activity prior to the alcohol there was an amplification of the spike activity during the post-alcohol phase.

Reports of withdrawal symptoms produced after very short drug exposures are much more common in the opiate literature. Martin and Eades (1961) detected antagonist-precipitated withdrawal effects in naive, spinal dogs after an 8-hr infusion of morphine. Similarly, a single injection of levorphenol in mice (Cheney & Goldstein, 1971) and a single injection of morphine in rats (Smits, 1975) both result in antagonist-precipitated withdrawal effects.

The Kindling Effect

1. General Description

The kindling effect refers to the discovery by Goddard (1967) that low-intensity, focal, electrical stimulation of the amygdala which initially

produces no epileptic response but does so with periodic presentation of the stimulation. Electrographic analysis has indicated that the periodic low-intensity stimulation first lowers the afterdischarge (AD) threshold until ADs are reliably elicited at the tip of the stimulating electrode (Racine, 1972a). With continued periodic elicitation of ADs the degree to which they generalize to other parts of the brain increases until mild motor automatisms are elicted. If even more stimulations are administered, the degree to which ADs generalize continues to increase until full motor seizures (MSs) are elicited by each stimulation (Racine, 1972a,b).[1]

The generality and permanence of the kindling phenomenon have been well established. Kindling has been demonstrated in rats (Goddard et al., 1969), mice (Leech, 1972), cats (Morrell, 1973), rabbits (Tanaka, 1972), primates (Wada & Sato, 1973) and frogs (Morrell, Tsuru, Hoeppner, & Morgan, 1975). The permanence of the change in neural function induced by periodic brain stimulation was first demonstrated by Goddard et al. (1969) who stimulated rats after a 12-week stimulation-free period and found a savings of about 90% in the number of stimulations required to elicit a full MS.

Kindling has been shown to occur in response to stimulation of the olfactory and limbic areas of the brain (Goddard et al., 1969), of extrapyramidal motor areas closely related to the limbic system (Goddard et al., 1969), and of some cortical areas (Racine, 1975). Since the amygdala has proved to be the most easily kindled structure, most studies of kindling have employed amygdaloid stimulation. However, regardless of the

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1 Due to the generally accepted terminology, overt and EEG components of a kindled seizure will be represented as MS and AD respectively. Previously, convulsions and EEG epileptiform activity represented these respective types of epileptic activity. Convulsive effects and seizures referred to both types of epileptic activity; the latter term also referred to increased seizure susceptibility.

site, stimulation must be presented periodically in order for kindling to take place. Racine, Burnham, Gardner, and Levitan (1973) and Goddard et al. (1969) indicated that there is a general inverse relationship between the number of stimulations required for kindling and the duration of the interval between stimulations. Goddard et al. found intervals of 24 hr or greater to be optimal and intervals of less than 20 min to be completely ineffective. Moreover, Morrell (1973) indicated that continuous stimulation not only did not produce kindling but retarded kindling significantly when distributed stimulation was subsequently administered.

Racine (1972a) has provided convincing evidence that the elicitation of ADs at the site of stimulation is a necessary prerequisite for kindling of MSs. Periodic stimulation maintained at a level below the AD threshold did not lead to the development of behavioural convulsions, nor did it reduce the number of supra-threshold stimulations later required for kindling. Moreover, the main electrophysiological correlate of the kindling process appears to be the degree to which ADs generalize from the site of stimulation to other neural structures (Racine, Gardner, & Burnham, 1972).

2. Assessment of Seizure Susceptibility with Kindled Seizures

Kindling has been employed as a model of learning (Goddard et al., 1969; McIntyre & Goddard, 1973), as a method of producing a functional lesion (McIntyre & Molino, 1972), as a model of epileptogenesis (Wada, Sato, & Corcoran, 1974; Pinel, Mucha, & Phillips, 1975), as an amnesic agent (McIntyre, 1970), and as a method for studying state-dependent learning (McIntyre & Reichert, 1971). However, one of its most promising

applications has been in the assessment of the convulsive and anticonvulsive effects of centrally active agents. The effects of agents on the duration of ADs and MSs elicited from various sites in the brain have been assessed (Babington & Wedeking, 1973).

The features of the kindling phenomenon which make it a valuable addition to the methods available for gauging the convulsive effects of centrally active agents have been discussed several times previously (Babington & Wedeking, 1973; Pinel, Phillips, & MacNeill, 1973; Racine, Livingstone, & Joaquin, 1975). The locus, frequency, intensity, and timing of the test stimulation are all under strict experimental control. Moreover, in the later stages of kindling the AD and MS duration of each animal is remarkably consistent from trial to trial. Pinel, Phillips, and MacNeill (1973) reported that following the development of full MSs, subsequent stages of seizure development are characterized by a gradual increase in the uniformity of seizures produced by successive stimulations. Eventually seizures were characterized by very low day-to-day variation in AD duration and MS duration and pattern. Thus, with these highly predictable seizures even agents with only subtle effects may be assessed reliably. Kindled seizures have been employed to assess the anticonvulsant effects of antecedent footshock (Pinel et al., 1973), various components of canabis sativa (Fried & McIntyre, 1973; Corcoran, McCaughran, & Wada, 1974), diazepam (Babington & Wedeking, 1973; Racine et al., 1975; Wise & Chinerman, 1974), phenobarbital (Babington & Wedeking, 1973; Wise & Chinerman, 1974), and a number of tranquilizers (Babington & Wedeking, 1973).

Not only are kindled seizures useful for assessing anticonvulsant effects but they also may be employed to detect convulsant effects.

Babington and Wedeking (1973), for example, noted that d-amphetamine prolongs the duration of kindled seizures. Similarly, Racine et al. (1975) found that diphenylhydantoin and procaine enhanced the duration and propagation of the AD.

Seizures kindled from different sites can be affected in different ways by certain centrally active agents. Babington and Wedeking (1973) assessed the effects of drugs on seizures kindled by stimulation of the neocortex and those from the amygdala. Since some drugs did not affect both types of kindled seizures in the same way, they were able to suggest mechanisms or sites of action. Racine et al. (1975) also noted the differential effects of various drugs on cortical and subcortically elicited kindled seizures.

Thus, on the basis of existing literature it appears that the kindling paradigm could prove to be an extremely useful tool for assessing the changes in seizure susceptibility following brief exposures to ethanol.

Recapitulation

Withdrawal-produced convulsive effects have been demonstrated with many techniques in a variety of laboratory animals. However, increases in susceptibility to experimentally-induced convulsions seem to provide a more sensitive measure of such withdrawal effects than do increases in the incidence of spontaneous withdrawal seizures. The use of such measures has indicated that the convulsive effects may be evident after an organism's first exposure to ethanol.

The kindling paradigm has proven to be an extremely useful tool for assessing changes in seizure susceptibility. Thus, in the present

experiments, an attempt was made to study the convulsive effects of a single dose of ethanol using the kindling method.

EXPERIMENT 1

One purpose was to determine anticonvulsive and convulsive effects of a single injection of alcohol. A second purpose was to demonstrate the use of kindled convulsions in the assessment of these changes in seizure susceptibility. By noting the differences in the kindled seizures elicited by stimulation at various times before and after an alcohol injection, the time course of the alcohol-produced changes in seizure susceptibility could be followed in individual animals.

METHODS

Subjects: The subjects were 15, 250-310 g male, black-hooded rats purchased from the Canadian Breeding Laboratories (La Prairie, Quebec). Throughout the experiment the subjects were housed singly in stainless-steel cages under a 12-hr-light - 12-hr-dark cycle, with ad libitum access to food (Purina Lab Chow) and water. The rats weighed an average of 415 ± 11 g (± standard error of the mean, s.e.) on the test day.

Electrodes and Surgical Treatment: A bipolar electrode was aimed at the median nucleus of the right amygdala of each subject. The electrodes were contructed of two 0.25 mm nichrome wires twisted together and insulated with Insul-X. The electrode tips were separated by 0.5 mm and scraped to expose a 0.5 mm length of stimulating surface.

The surgery was conducted in accordance with standard stereotaxic technique under combined sodium pentobarbital (30 mg/kg) and chloral hydrate (125 mg/kg) anesthesia. The electrodes were implanted with a

Krieg stereotaxic instrument at coordinates of 1.5 mm posterior to Bregma, 1.5 mm lateral to the midline, and 8.8 mm below the dura. Following surgery each animal was routinely injected with 0.2 cc of pentylenetetrazol and 0.2 cc of pennicillin. The same dose of pennicillin was administered on each of two subsequent recovery days.

Histology: At the termination of the experiment all animals were asphyxiated with carbon dioxide and their brains were removed and stored in a buffered formalin solution for at least 1 week, at which time they were embedded in paraffin and sectioned at 20 microns. The sections were stained according to a modification of the Kluver-Bararra technique in order to verify the electrode placements.

Injection solutions: The ethanol was prepared by mixing 95% ethanol with 0.9% saline to produce a 20% by volume ethanol solution. These solutions were always prepared 24 hr prior to the injections and were stored at $4^{o}C$ until their use.

Stimulation and Recording: Tests were conducted in a 25 x 25 x 40 cm clear Plexiglas chamber fitted with a grid floor and housed in a grounded Faraday cage. Electrographic activity was monitored by a Grass 78B EEG polygraph through low noise, shielded recording leads. During stimulation the recording leads also served to deliver current across the electrode tips. The 1.0-sec, 400-uA (RMS) current, passed through a switching circuit which isolated the polygraph amplifiers during stimulation to reduce the post-stimulation interference in the recording channel.

Before each test session, each animal was allowed to acclimatize to

the leads and test chamber. The EEG activity was then recorded for 45 sec prior to current delivery and for 60 sec after the last spike of the AD. MSs elicited by the stimulation were classified according to the 5 stages of epileptogenesis described by Racine (1972b): 1) facial movements only; 2) facial movements and head-nodding; 3) facial movements, headnodding, and forelimb clonus; 4) facial movements, head-nodding, forelimb clonus, and rearing; and 5) facial movements, head-nodding, forelimb clonus, rearing, and loss of equilibrium.

Kindling Procedure: Recording commenced 21 days after surgery. On the first day EEG activity was recorded for 10 min to check the connectors and recording electrodes and to habituate the subjects to the apparatus. Kindling began the following day. Three stimulations were given each day, 5 days each week for three weeks. The stimulations were given no less than 0.5 hr and no more than 18 hr apart. The animals were then switched to a stimulation regiment which involved one stimulation per day for 17 days. At that point, all animals were consistently exhibiting class 5 MSs except for 5 animals consistently displaying class 4 MSs. The MS and AD duration of seizures elicited in each animal by the last four stimulations of the series never differed by more than 25% on consecutive days.

Experimental Procedure: Testing began on Day 18 with each rat receiving 18 stimulations, one every 1.5 hr. Following three baseline test stimulations the animals were assigned to two groups matched as much as possible for AD and MS duration and body weight. The injections of saline and ethanol were given 30 min prior to the fourth stimulation. The ethanol group (n = 8) received an i.p. injection of 20% ethanol solution containing

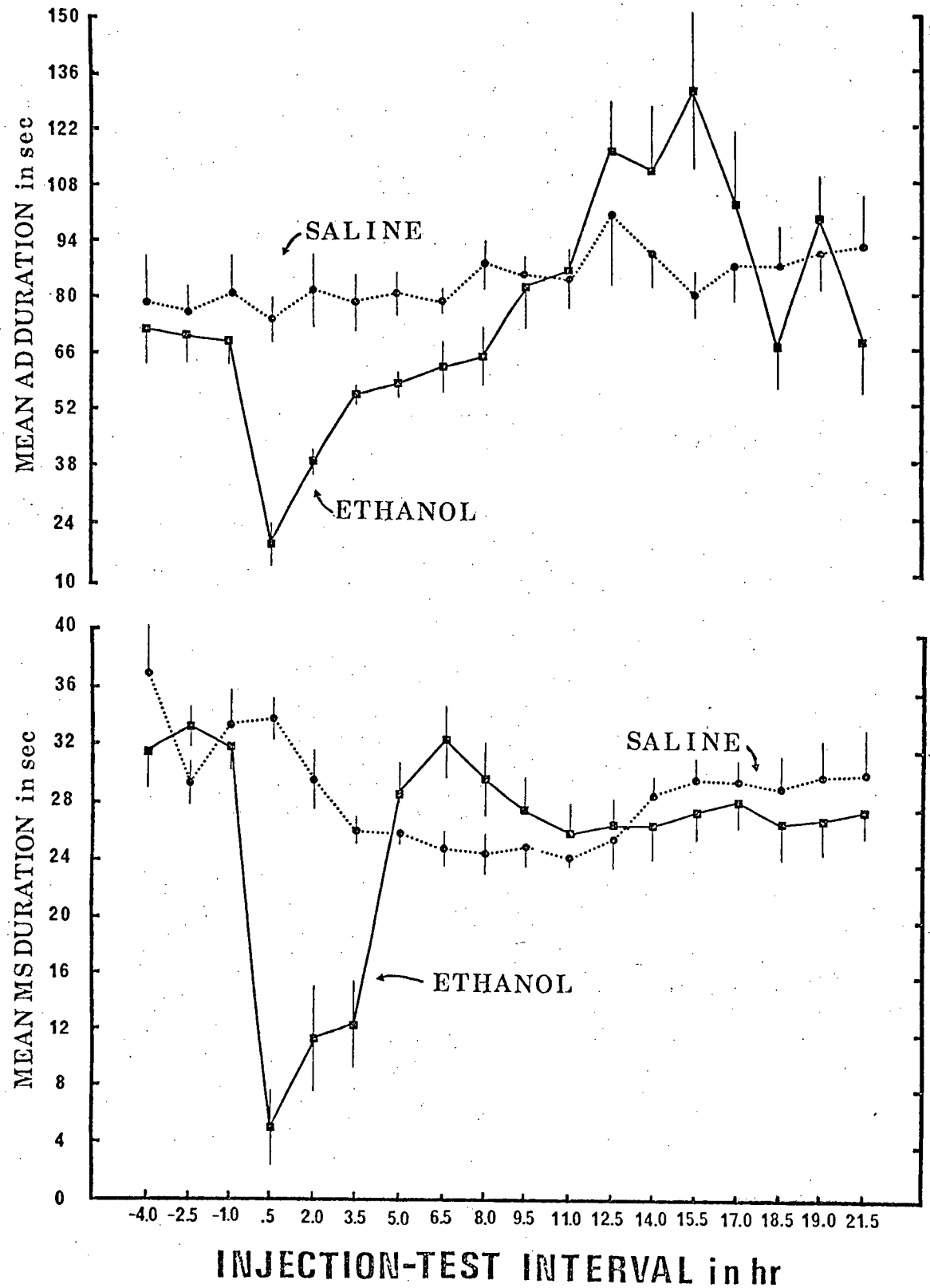
2.0 g/kg of absolute alcohol, whereas the control group (n = 7) received an i.p. injection of a similar quantity of saline. The quantity consisted of 12.6 ml/kg of fluid. The dose of 2.0 g/kg was chosen since this level produced mild ataxia but no anesthesia when given to pilot animals.

RESULTS AND DISCUSSION

The results are summarized in Fig. 1. The durations of AD and MSs elicited by stimulations administered immediately after the alcohol injection were reduced appreciably, but following this suppression there was a transient but marked increase in the AD duration.

The results of the overall analysis of variance and the tests of simple main effects (Kirk, 1968) are presented in Appendix A. These justified the use of multiple comparison tests. Tukey "A" tests carried out on the means of the ethanol group indicated that the mean AD duration 0.5 hr following the alcohol injection was significantly shorter, and the mean AD duration at 15.5 hr was significantly longer, than the ADs elicited by the three baseline stimulations in the same animals (Tukey's test, HSD = 36.2 $p<.05$). Moreover, duration of the ADs elicited 0.5 hr after the ethanol was significantly less than for all other test stimulations administered following the alcohol; while the duration of the ADs elicited 15.5 hr after the injection was significantly greater than all other ADs elicited in the ethanol subjects except the ones at 12.5, 14.0, 17.0 and 21.5 hr (Tukey's test, HSD = 36.2, $p<.05$). The pattern of significance was also illustrated by the results of between-group comparisons; the ethanol subjects had significantly shorter ADs 0.5 and 2.0 hr after the injection and significantly longer ADs at 15.5 hr (Tukey's test, HSD = 36.2, $p<.05$). There was no

Fig. 1 Mean (± s.e.) AD (top panel) and mean MS (bottom panel) duration of kindled seizures elicited in the rats of the two experimental groups at the 18 injection-test intervals in Experiment 1.


MEAN AD DURATION in sec
150
136
122
108
94
80
66
52
38
24
10
SALINE
ETHANOL
MEAN MS DURATION in sec
40
36
32
28
24
20
16
12
8
4
0
SALINE
ETHANOL
-4.0 -2.5 -1.0 .5 2.0 3.5 5.0 6.5 8.0 9.5 11.0 12.5 14.0 15.5 17.0 18.5 19.0 21.5
INJECTION-TEST INTERVAL in hr

significant variation in the mean AD durations over the 18 stimulations in the control conditions. In individual experimental animals the increase in duration of the AD appeared at points between 11.0 and 20.0 hr after the alcohol injection. These increases, however, were not consistently present over this period; they occurred interspersed with ADs of regular duration with the primary concentration of the long ADs occurring between 12.0 and 15.5 hr.

Similar multiple comparison tests were carried out on the mean MS durations. Test stimulations 0.5, 2.0, and 3.5 hr after the ethanol injection evoked shorter MSs than did any of the three pre-injection baseline stimulations (all Tukey's test, HSD = 8.56, $p < .05$). Comparisons of the means of animals receiving alcohol and those receiving saline were also significant at 0.5, 2.0 and 3.5 hrs (Tukey's test, HSD = 9.15, $p < .05$). At no point was the MS of the group receiving ethanol significantly longer than for the corresponding control points, and there were no mean MS durations following the ethanol injection that were significantly longer than the mean MSs of the three baseline test stimulations. However, at 6.5 hr almost every experimental animal had a longer MS than those of the control animals.

The duration of the MSs elicited in the saline controls also varied over the 18 stimulations. Multiple comparisons indicated that the first mean MS was significantly longer than the mean MS at 3.5, 5.0, 6.5, 8.0, 9.5, 11.0, and 12.5 hr (all Tukey's test, HSD = 9.15, $p < .05$).

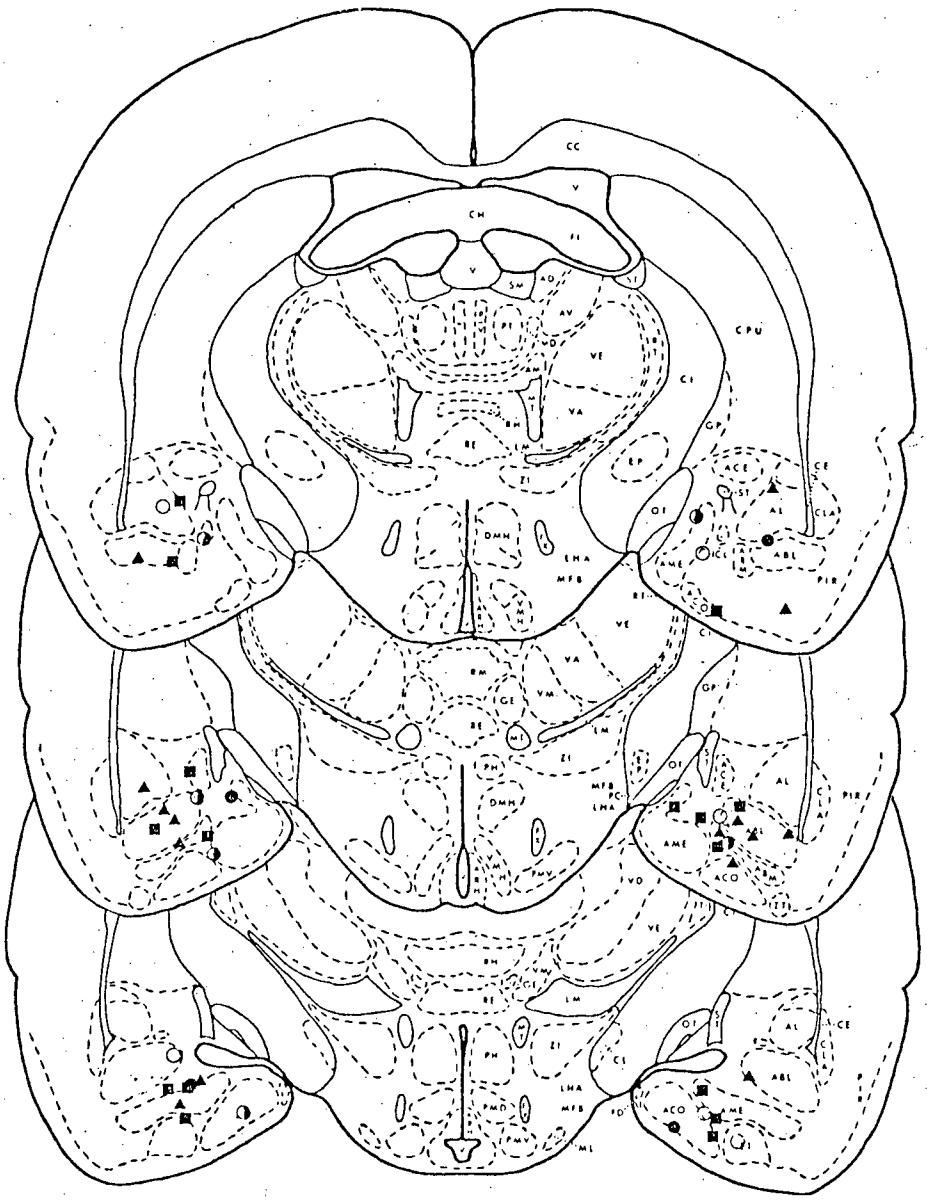
The MS class and latency to MS onset were also monitored. In the experimental group the three baseline test stimulations elicited class 4 and 5 MSs with MS latencies being less than 1 sec for all but one. The MS was blocked in all but three experimental animals at the 0.5-hr interval.

These three animals experienced a seizure pattern of class 3 or less with a longer seizure latency. At the 2.0-and 3.5-hr intervals all animals exhibited MSs but they were of a lower class and had longer latencies. The MS class and MS latency measures proved to be of little value in detecting any increases in seizure susceptibility possibly because of "ceiling and basement' effects, respectively.

Histological examination revealed that most electrodes had, in fact, been implanted in the medial amygdala (see Fig. 2). In one subject the tip was located in the pyriform area; however, this animal's behaviour could not be distinguished from the others. Attempts to correlate the placements with the behaviour of individual rats were unsuccessful.

The relative stability of seizures in the control animals over the 18 stimulations reveals the potential of the kindling paradigm for tracing the course of the changes in seizure susceptibility produced by the alcohol. The ADs of most individual animals were very similar to one another during the regiment of 1.5-hr-interval stimulations. This was also the case for the MSs and although the mean duration of the MSs changed significantly over the course of the 18 stimulations, the change was gradual and consistent in all animals. Against the stable control baseline the time course of changes in seizure susceptibility are easy to detect. Although kindled seizures have been previously used to assess convulsive and anticonvulsive effects, they have never been used, as in this experiment, to follow the time course of these effects in individual subjects. Although distributed stimulations are necessary for kindling, once kindled, rats will respond consistently to stimulation administered at relatively short intervals. This is not the case, however, for rats repeatedly stimulated with other convulsive agents.

Fig. 2 Electrode placements of subjects in the four experiments. Each symbol represents one rat in the experiments given at the right. Atlas diagrams are from Pellegrino and Cushman (1967).


5.4
5.0
4.6
▲ Exp 1
○ Exp 2
◑ Exp 2&3
● Exp 3
■ Exp 4

For example, experiments in our laboratory revealed that convulsions elicited in rats by electroconvulsive stimulation administered every hr undergo drastic decreases in the severity and duration, and result in heavy subject loss due to death and convulsion-produced injuries (Van Oot & Pinel, unpublished observations). Thus, in comparison to other methods, kindled seizures provide a valuable method of following the time course of the effects in individual animals.

The time course of the changes in seizure susceptibility suggests that the anticonvulsive effects were present during the period of high blood ethanol levels and the convulsive effects during a period immediately following alcohol metabolism. Owens and Marshall (1955) found that 2.0 g/kg administered to 300 to 400 g rats is quickly absorbed into the blood and is metabolized in approximately 9 hr. Thus, the present results suggest that ethanol is a potent anticonvulsive agent. Since the convulsive effects seem to have occurred following the metabolism of the alcohol they appear to be true withdrawal-produced effects.

EXPERIMENT 2

Comparisons with the results of Owens and Marshall (1955) suggest that the anticonvulsive and convulsive effects observed in Experiment 1 were related to the presentation and withdrawal of alcohol respectively. The purpose of Experiment 2 was to replicate the results of Experiment 1 and to relate the anticonvulsive and convulsive effects to changes in blood ethanol levels.

The methods were similar to those of Experiment 1 except for the following innovations. First, only those kindled subjects that consistently displayed ADs and MSs of stereotyped duration and pattern were tested. Second, a higher dose of alcohol was administered in an attempt to produce greater changes in susceptibility. Third, the test stimulations were administered at intervals of 3.0 hr rather than 1.5 hr in an attempt to eliminate shifts in the baseline. Fourth, each animal was tested under both the experimental and control conditions so that a particular rat could act as its own control.

METHODS

Subjects: The subjects were 18 male rats similar to those used in Experiment 1. They were maintained, implanted, and kindled as in Experiment 1. In the present experiment, however, animals were not in the experiment proper unless they reached the criterion of seizure stability employed by Pinel, Phillips, and MacNeil (1973). The subjects were required to exhibit 10 consecutive class 5 MSs with no more than 20%

variation of the MS and AD durations elicited on consecutive days. Six of the 12 subjects that reached this criterion did so after 18 daily stimulations; the other six after 25 days.

Ethanol Assays: The blood ethanol levels were determined according to a procedure similar to that of Baker, Alenty, and Zach (1969) and Roach and Creaven (1968). Blood was withdrawn from the tail vein 5 min after seizure elicitation. Two 30 ul samples of blood were taken, each drawn into calibrated 75 ul capillary tubes. Fifteen ul of 5% zinc sulfate and 15 ul of 1 normal sodium hydroxide, both cooled to $4^{o}C$, were immediately added to the blood. The tubes were sealed and stored at $4^{o}C$ for at least 24 hr but no more than 48 hr before assaying.

Prior to assaying the blood sample the capillary tubes were centrifuged at 10,000 x gravity for 10 min. A 1.0 ul quantity of clear supernatant was then withdrawn with a Hamilton syringe from the top of the tube and injected into a Perkins-Elmer 900 programmable gas-liquid chromatograph. The helium carrier gas passed through a column packed with carbowax and maintained at a temperature of $100^{o}C$. The carrier gas then passed over an ionizing hydrogen flame where the presence of ethanol was detected and recorded on a chart recorder. The amount of ethanol was calculated by making comparisons to identical external standards of known ethanol concentration. Each of the two blood samples was analyzed twice.

Experimental procedure: Testing commenced the day after the criterion of stability was met. This occurred after the 18th daily stimulation for six animals and after the 25th for the other six. Over the 33-hr testing period each animal was stimulated 12 times, once every 3.0 hr. Thirty min

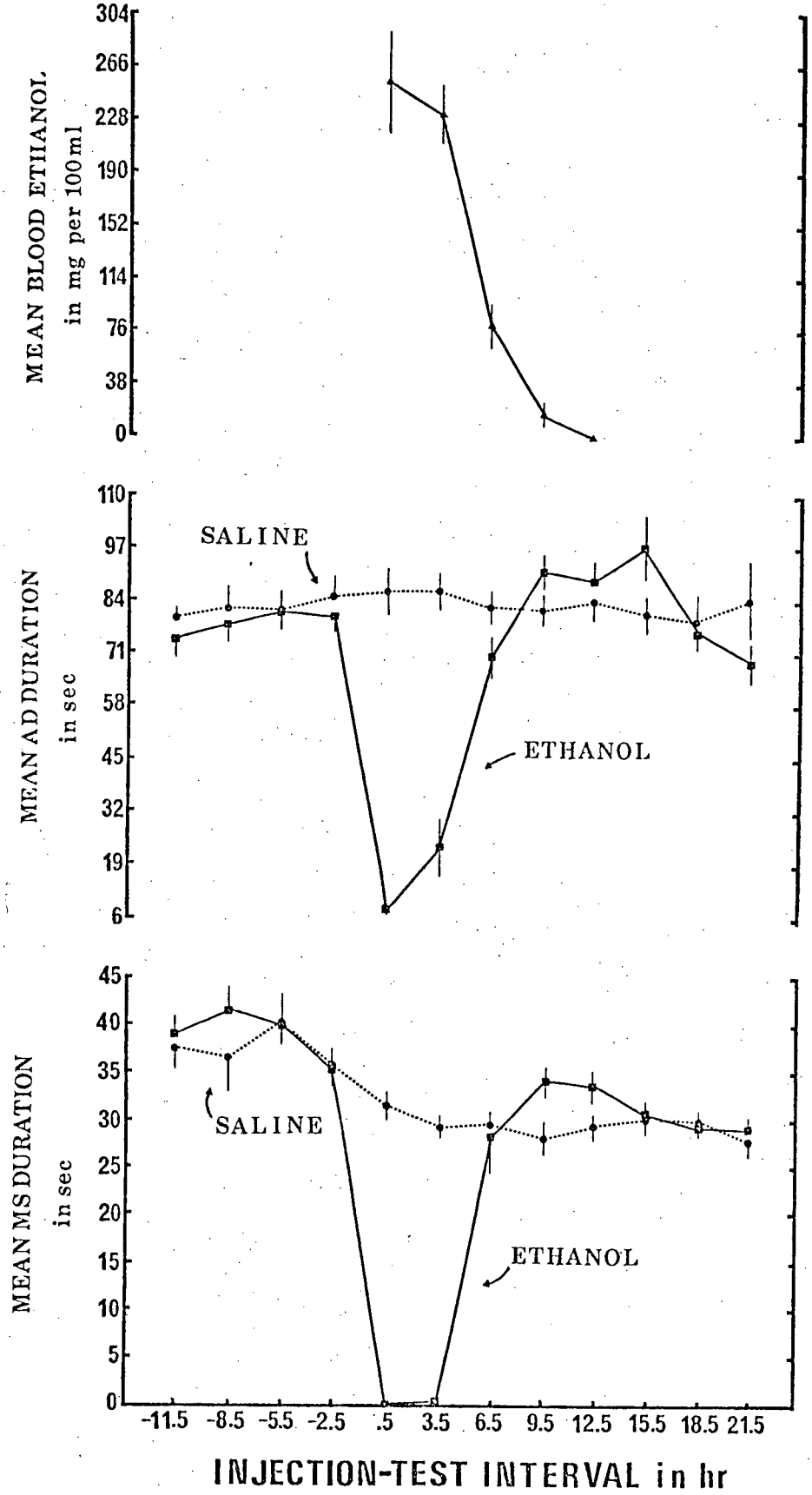
before the fifth stimulation six rats received an i.p. injection of 2.5 g/kg of alcohol and six received an equivalent volume of saline (15.7 ml of fluid per kg). After this testing period the animals were returned to the daily stimulation regimen for 12 days before being tested as before over another 33-hr session. In this second session animals that had received saline in the first test received ethanol, and vice versa. During both test sessions two samples of blood were taken from every animal five min following the cessation of the response evoked by each of the 5th through to the 11th test stimulations.

RESULTS AND DISCUSSION

The major results of Experiment 2 are illustrated by Fig. 3. The pattern of anticonvulsive and convulsive effects was comparable to that found in Experiment 1. The results of the blood ethanol analyses confirmed the relation of the anticonvulsive and convulsive effects with the presence and absence of high blood ethanol levels, respectively. A summary of the overall analysis of variance of these results is presented in Appendix B.

The alcohol-produced biphasic effects on AD duration similar to those observed in Experiment 1. A priori multiple comparison tests indicated that the mean AD duration elicited at 0.5 and 3.5 hr was significantly shorter, and the mean MS duration elicited at 15.5 hr was significantly longer than the mean AD of the fourth baseline stimulation (Dunn's test, critical difference = 11.5, $p<.05$). Comparisons between the ethanol and saline conditions at each interval also revealed the biphasic effects of alcohol. There was a significant difference between the means at 0.5,

Fig. 3 Mean (± s.e.) concentration of blood ethanol (top panel), AD duration (middle panel), and MS duration (bottom panel) elicited by the test stimulations at the 12 injection-test intervals in Experiment 2.


MEAN BLOOD ETHANOL
in mg per 100 ml
304
266
228
190
152
114
76
38
0
MEAN AD DURATION
in sec
110
97
84
71
58
45
32
19
6
SALINE
ETHANOL
MEAN MS DURATION
in sec
45
40
35
30
25
20
15
10
5
0
SALINE
ETHANOL
-11.5 -8.5 -5.5 -2.5 .5 3.5 6.5 9.5 12.5 15.5 18.5 21.5
INJECTION-TEST INTERVAL in hr

3.5, and 15.5 hr (Dunn's test, critical difference = 13.59, $p < .05$) while at 9.5, 12.5, and 18.5 hr there were no significant differences between the conditions. There were no systematic changes in the mean AD duration in the control conditions. These AD data thus replicate the results and conclusions of Experiment 1.

The changes in the MS duration were also similar to those observed in Experiment 1. A priori comparisons of means in the ethanol condition indicated that the mean durations of the MSs elicited 0.5 and 3.5 hr after the ethanol injection were significantly less than the mean duration of the MSs evoked by the fourth baseline test stimulation (Dunn's test, critical difference = 5.07, $p < .05$). Comparisons between the means of the ethanol and saline conditions at each interval revealed that the MS durations of the ethanol group were significantly shorter at 0.5 and 3.5 hr and significantly longer at 9.5 and 12.5 hr after the ethanol injection (Dunn's test, critical difference = 4.99, $p < .05$). In addition, the gradual changes in the MS duration observed in the control condition of Experiment 1 were also observed in the present experiment. The durations of MSs elicited by the first stimulation in the saline condition were significantly different than the MS duration at 0.5, 3.5, 6.5, 9.5, and 21.5 hr (Dunn's test, critical difference = 5.07, $p < .05$).

Thus, despite the procedural differences the MS data too were comparable to those of Experiment 1. The results of the statistical analyses differed in one respect but this was not a reflection of differences in the data. In both experiments the MSs elicited after the anticonvulsive effect were longer than those elicited in the saline condition; however, this difference was not found to be significant in Experiment 1 without the use of a priori statistical tests.

The results of the blood ethanol assays confirmed that the increases in AD and MS durations were true withdrawal-produced effects. Significant increases in AD duration were observed at 15.5 hr and MSs were significantly longer at 9.5-and 12.5-hr intervals even though there was little or no ethanol in the blood over this period. Differences in the blood ethanol levels observed between animals at particular intervals were not systematically related to differences in MS or AD duration.

The electrode placements in the animals of Experiment 2 were similar to those of Experiment 1 (see Fig. 2). It is apparent that all electrodes were in the medial amygdala. No systematic relation between electrode placement and behavior of an individual animal could be found.

EXPERIMENT 3

The results of Experiment 1 and 2 suggest that the metabolism of an organism's first dose of ethanol can have convulsive effects. However, there is another possible interpretation of the convulsive effects. They may simply be an artifact of the testing procedure rather than of alcohol withdrawal per se. It is well-established that kindled seizures can have suppressive effects on subsequent kindled seizures (Mucha & Pinel, 1975; Pinel, Phillips, & Deol, 1974; Racine, 1972b). Thus, the longer seizures observed during the withdrawal period may have simply been a result of ethanol's anticonvulsive effects on earlier seizures during the intoxication period: alcohol may have reduced the suppressive effects of earlier upon later seizures. The purpose of Experiment 3 was to determine if the long ADs and MSs observed during the withdrawal period were the direct result of the alcohol's anticonvulsant effects or the indirect result of the repeated testing procedure. Each subject was stimulated at only one interval following each injection of alcohol or saline.

METHODS

Subjects: The subjects were the 10 rats that completed Experiment 2, and the 6 rats that failed to meet the stability criterion by the 25th daily stimulation. These 6 rats received daily stimulation while the other 10 were being tested in Experiment 2. By the end of this period all 6 rats had reached the criterion of stability consisting of 10 consecutive class 5 MSs with less than 20% variation in MS and AD duration on consecutive

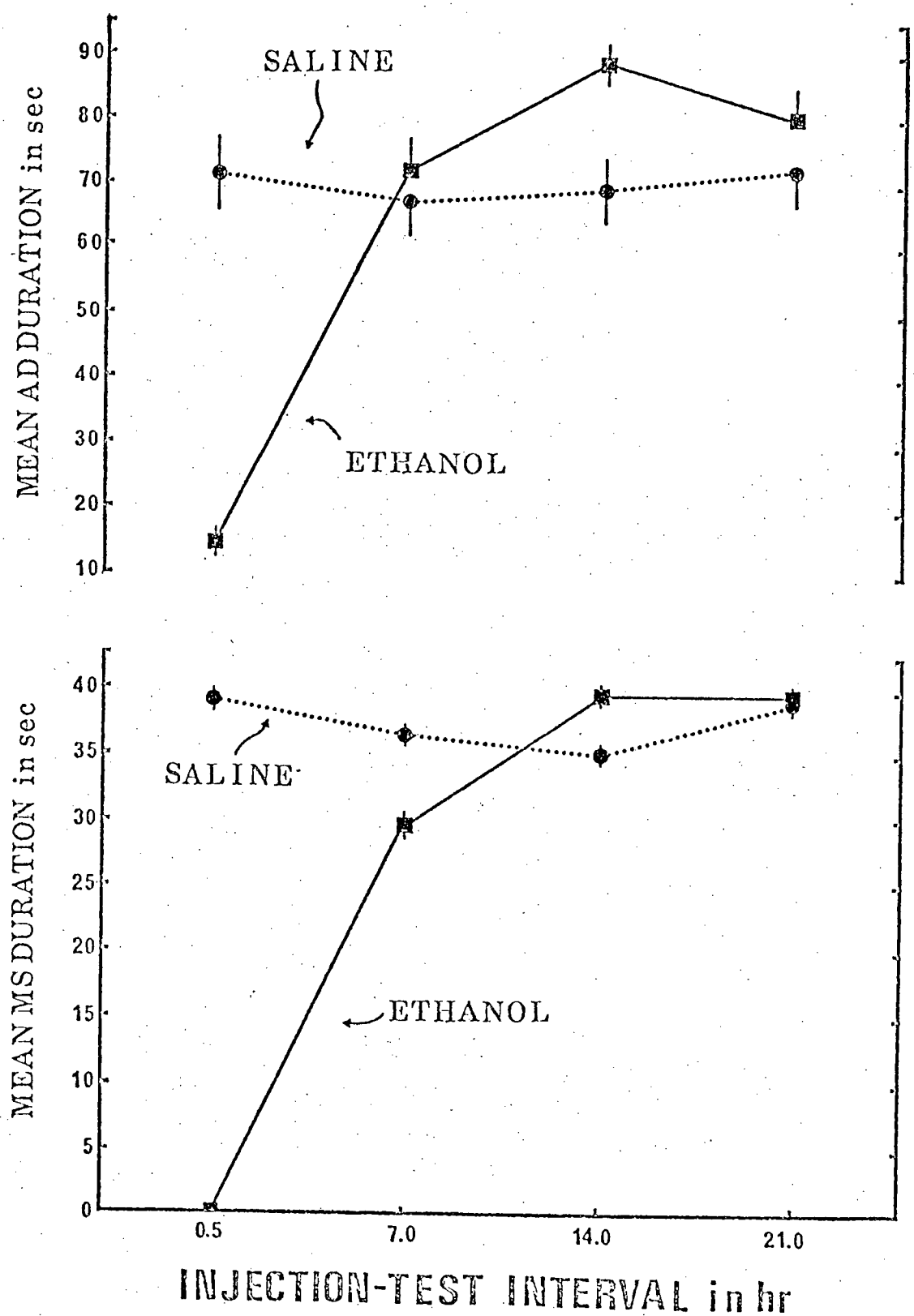
days. All 16 animals were not stimulated in the 4 weeks between Experiments 2 and 3. At the beginning of Experiment 3 they weighed between 400 and 560 g.

Experimental Procedures: During the course of the experiment each rat was stimulated at the same time each day for 48 consecutive days. The first 14 days served as a baseline period during which 12 animals exhibited a high degree of stability consisting of at least 5 consecutive class 5 MSs with no more than 20% difference in the durations of either MSs or ADs elicited on consecutive days. At the end of this 14-day period the 12 animals were divided into two equal groups matched according to AD duration. The remaining four rats were not included in the present experiment. On days 15, 17, 19, and 21 one group of six animals received an i.p. injection of 2.5 g/kg of ethanol and one group received an equivalent volume of saline (15.7 ml of fluid per kg). These injections were administered either 0.5, 7.0, 14.0, or 21.0 hr before each daily stimulation. A second series of injections was administered on days 36, 38, 40, and 42; however, during this second series the animals that had previously received the ethanol injections received the saline and vice versa. In all other respects the tests were the same. The order of the four injection intervals was determined according to a 4 x 4 latin square.

RESULTS AND DISCUSSION

The results are presented in Fig. 4. Even though a different experimental design was employed in the present experiment, the results were similar to those of the initial experiments; anticonvulsive and convulsive

Fig. 4 Mean (± s.e.) AD (top panel) and mean (± s.e.) MS (bottom panel) duration elicited at the four injection-test intervals in Experiment 3.


MEAN AD DURATION in sec
90
80
70
60
50
40
30
20
10
SALINE
ETHANOL
MEAN MS DURATION in sec
40
35
30
25
20
15
10
5
0
SALINE
ETHANOL
0.5
7.0
14.0
21.0
INJECTION-TEST INTERVAL in hr

effects were observed following a single injection of alcohol. A summary of the overall analysis of variance of the MS and AD durations is presented in Appendix C.

The changes in the mean AD duration was similar to that observed in Experiments 1 and 2: a priori multiple comparisons between means of the experimental condition and of the control indicated that the ethanol injection reduced significantly the duration of ADs elicited 0.5 hr after the alcohol and significantly increased the duration of those elicited at 14.0 hr (Dunn's test, critical difference = 13.05, $p<.05$). Comparisons between the mean MS durations of the various ethanol and saline conditions indicated that at 0.5 and 7.0 hr the mean MS duration of the subjects in the ethanol condition was significantly less than that of the subjects in the saline condition (Dunn's test, critical difference = 6.77, $p<.05$). The mean difference between the data of subjects in the ethanol and in the saline conditions tended towards significance at 14.0 hr. At this time interval 9 of the 12 animals exhibited a longer MS following the ethanol injection than following the saline injection.

The location of the electrode tips of the subjects are presented in Fig. 2. All electrodes were in the medial amygdala and, as in previous experiments, no systematic relation was found between location and changes in seizure susceptibility of individual animals.

The results of this experiment, therefore, provide strong evidence that the withdrawal from a first exposure to ethanol can have convulsant effects. This convulsant effect does not appear to be due to a post-suppression "rebound" effect of the earlier anticonvulsive effects of alcohol. Alcohol resulted in an increase in mean seizure duration even when only a single

post-injection stimulation was used to assess the effect.

EXPERIMENT 4

The purpose of Experiment 4 was to confirm the results of Experiment 3 and establish their generality by administering the ethanol intragastrically rather than intraperitoneally as in the first three experiments. Because of its rapid absorption into the blood, intraperitoneally administered ethanol may produce qualitatively different results than those following routes of administration with lower rates of absorption (cf. Kalant, 1961).

METHOD

Subjects: The subjects (N=19) and surgical and kindling procedures were similar to those employed in Experiment 1. By the 25th daily stimulation the three rats that had not reached the criterion of 10 consecutive days with less than a 25% difference on consecutive days in either MS or AD duration were excluded.

Experimental Procedure: The experiment commenced on the day following the 25th daily stimulation and lasted for seven days. Each day each animal received one stimulation at the same time of day. On days 1, 3, 5, and 7 each rat was exposed to one of four experimental conditions, 2.5 g/kg of ethanol administered by gavage at either 11.0, 14.0, or 17.0 hr before the stimulation or an equivalent volume of saline (15.7 ml/kg) administered 14.0 hr before the stimulation. The order in which these four treatments were presented was determined by randomly assigning 4 rats to each row of a 4 x 4 latin square.

RESULTS AND DISCUSSION

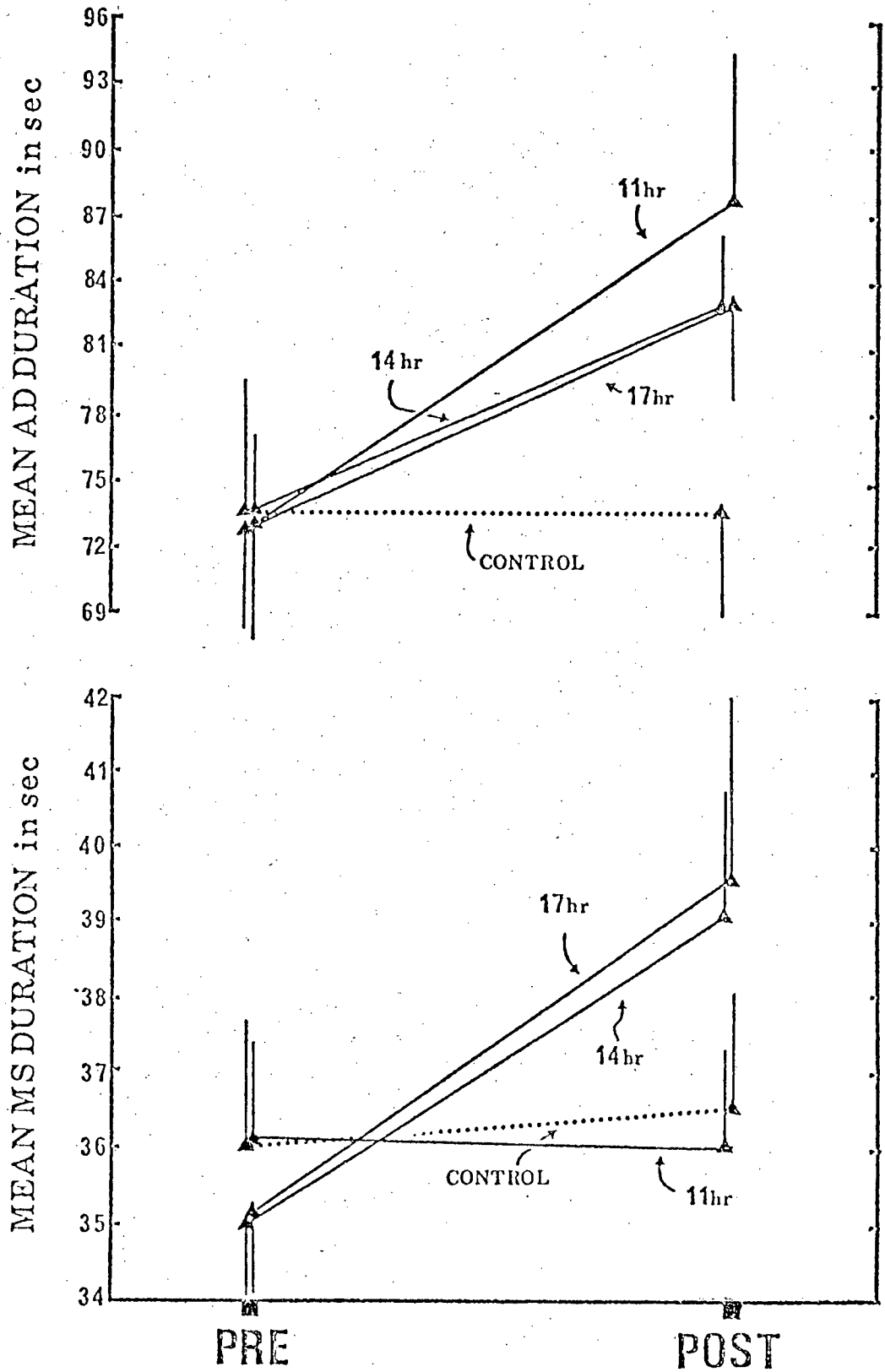
The results are summarized in Fig. 5. Only long ethanol-test intervals were looked at in the present experiment and changes in seizure susceptibility consistent with those of previous experiments were found. Thus, intragastrically administered as well as intraperitoneally administered ethanol results in increases in susceptibility to kindled seizures several hr after the ethanol administration. A summary of the overall analysis of variance for AD and MS durations is presented in Appendix D.

A priori comparisons indicated that the mean AD duration elicited at 11.0 hr following the ethanol intubation was significantly longer than the mean AD duration following a similar saline intubation (Dunn's test, critical difference = 10.38, $p < .05$). The AD duration at 14.0 and 17.0 hr following the intubation was not statistically different than the mean duration elicited following the saline.

Multiple comparisons of the mean MS durations of the experimental conditions to that of the control condition failed to reveal any statistical differences (Dunn's test, critical difference = 3.73, $p < .05$). In previous experiments the MS duration increases were not as reliable as those of the AD but they have nevertheless been present. Since only three relatively similar intubation-test intervals were used the lack of an appreciable change in the MS duration of the present experiment may have been due to a failure to employ appropriate test intervals.

The electrode tips of all the rats in the present experiment were located in the medial amygdala (see Fig. 2). No systematic relation between

Fig. 5 Mean AD (top panel) and mean MS (bottom panel) duration elicited by the test stimulation before and after an intragastric injection of ethanol or saline. The vertical line at each point is the s.e.


MEAN AD DURATION in sec
96
93
90
87
84
81
78
75
72
69
11hr
14 hr
17hr
CONTROL
MEAN MS DURATION in sec
42
41
40
39
38
37
36
35
34
17hr
14hr
CONTROL
11hr
PRE
POST

behaviour and electrode placement was found.

There are two major findings of the present experiment. First, the results demonstrate convulsive effects several hr after an intragastrically administered dose of ethanol. This indicates that the increased seizure susceptibility found in the previous experiments was not specific to intraperitoneally administered ethanol. There are a number of differences between these two methods of injecting alcohol. For example, ethanol administered intraperitoneally is absorbed very rapidly and produces relatively high blood ethanol levels, while intragstrically given ethanol is absorbed slower and reaches a much lower blood level (cf. Wallgren, 1970). Thus, the convulsive effect noted in the present experiments is not a result of either the rapid rate of ethanol absorption or the higher blood ethanol levels that are specific to the intraperitoneal route of ethanol administration.

The second major finding of the present experiment was that convulsive effects after a brief ethanol exposure were demonstrated with a design using only a single test stimulation after each intubation of ethanol. This replicates the results of Experiment 3 and confirms the conclusion that the increased susceptibility to kindled seizures found in the first two experiments was not an artifact of the repeated testing procedure employed.

GENERAL DISCUSSION

The primary purpose of the present investigation was to study the convulsive effects produced in naive organisms following a single brief exposure to ethanol. An additional purpose was to describe a method of using kindled seizures to measure the time course of changes in seizure susceptibility. Hence, the discussion will begin with a consideration of these two aspects of the present investigation. This section will continue with a discussion of the following topics: other effects of brief alcohol exposure, hangover, the relation of pathological effects of alcohol exposure to withdrawal seizures, and the concept of physical dependence.

Convulsive Effects of a Single, Brief Exposure to Ethanol

The major finding of the present experiments was a significant increase in the duration of kindled seizures elicited several hr after an organism's first injection of ethanol. Thus, these results confirm the previous demonstrations of increases in seizure susceptibility following a single dose of ethanol (McQuarrie & Fingl, 1958; Goldstein, 1972). The study of these convulsive effects extended those of these previous investigations in three different ways. First, the generality of these effects was illustrated. The demonstration of increased seizure susceptibility following a single brief exposure to ethanol is not peculiar to either the laboratory mouse or to convulsions elicited by handling or pentylenetetrazol (McQuarrie & Fingl, 1958; Goldstein, 1972). Second, an increase in seizure susceptibility following a single injection of ethanol was demonstrated using two entirely

different experimental designs. In addition to assessing seizure susceptibility at multiple fixed intervals following the injection, the method used by both McQuarrie and Fingl (1958) and Goldstein (1972), a design with only a single test after each alcohol injection was used. Therefore, changes in seizure susceptibility several hr after the alcohol injection are not simply artifacts of the multiple-assessment procedure. Third, the present experiment provided the first clear demonstration that the convulsive effects following brief exposure are triggered by the metabolism of the ethanol rather than the presence. At low concentrations ethanol may have direct convulsive effects that will decrease the amount of current required to elicit an AD with focal stimulation of the cortex and will prolong the AD once it is elicited (Kalant, 1970). In Experiment 2 increased seizure susceptibility was clearly present after all the ethanol had been metabolized, thus precluding the convulsive effects of ethanol as the basis of these increases. Goldstein (1972) had previously related blood ethanol levels to increases in seizure susceptibility following single brief exposures; however, in her studies increases in susceptibility to handling convulsions peaked while some ethanol was still present in the blood.

Thus, the results of the present experiments provide clear support for the generality of withdrawal-produced convulsive effects following brief exposures of ethanol to naive organisms. The implication of these findings is that the process responsible for the severe withdrawal-produced convulsive effects following chronic alcohol exposure in humans begins to develop with the first exposure of ethanol. Consistent with this view are the reports of epileptic effects in nonalcoholic epileptics (Victor, 1968;

Lennox, 1941) and in young healthy persons (Nagy, et al., 1973) after brief ethanol exposures.

Although it is strongly suggested that the convulsive effects after the anticonvulsive effects of the alcohol are withdrawal-related, the present experiments have not precluded the possibility that part of the convulsive effect may be produced indirectly. For example ethanol exposure produces an increase in blood pH (Wolfe & Victor, 1971) which in the epilepsy literature has been demonstrated to increase the susceptibility to audiogenic seizures (Goldman, Lisak, Matz, &mDavidson, 1970). Thus, the increases in seizure susceptibility may be due to a mild state of alkalosis produced by the brief ethanol exposure.

Kindled Seizures and the Assessment of Seizure Susceptibility

The present investigation demonstrated a number of features of kindled seizures which make them extremely useful for assessing changes in seizure susceptibility. For example, since low-intensity stimulation will eventually elicit seizures which vary little from day-to-day agents having only subtle effects on seizure susceptibility can be assessed without having to use large numbers of subjects. Stability was apparent not only when stimulation occurred every 24 hr, but also when it occurred at intervals as short as 1.5 hr. Another feature of kindled seizures illustrated by the present studies was their potential for repeated testing. After an animal has been kindled, seizures can be reliably elicited even after long rest periods (cf. Goddard et al.,

1969), and they rarely cause fatalities or injuries. In the present studies subjects were repeatedly tested in every experiment and the same animals were used in Experiments 2 and 3. An additional feature is their sensitivity to both increases and decreases in seizure susceptibility. This was clearly evidenced by the detection of both anticonvulsive and convulsive effects of alcohol exposure.

Thus, the present data, confirm and extend a number of previous demonstrations that kindled seizures could be used to assess seizure susceptibility (cf. Babington & Wedeking, 1973; Pinel et al., 1973; Racine et al., 1975). The major contribution of the present study was the finding that kindling may be used to accurately follow the time course of a convulsive or anticonvulsive agent in a single animal. Typically, most studies have measured the seizure susceptibility at only a single interval after each administration of a particular agent. This has likely resulted from the early report that interstimulation intervals of 24 hr were the most effective for kindling (Goddard, et al., 1969). Thus, few experimenters have studied the effects of kindled seizures elicited by stimulation separated by short intervals; the type of stimulation necessary for the study of time courses of changes in seizure susceptibility lasting only a few hr. The present data also illustrate the potential of kindling for understanding the mechanisms underlying changes in seizure susceptibility. For example, the lack of a correlation between the increases in mean AD duration and in mean MS duration indicates that alcohol withdrawal does not have nonspecific convulsive effects; otherwise, the AD and MS durations should have been similarly affected. The data also suggest that the convulsive effect

of the ethanol acts initially on the area of the brain responsible for motor function. The first successful use of kindled seizures to similarly study mechanisms of drug actions was by Babington and Wedeking (1969, 1973). By studying differential effects of centrally active agents on amygdaloid, septal and cortical kindling they concluded that the action of some drugs is primarily in one area of the brain and not in others. The conclusions based on these differential influences on kindling were highly consistent with other data specifying sites of the brain where the individual drugs act. Thus, the present data suggest that the convulsive effects following the metabolism of ethanol may be subjected to a similar type of analysis of mechanisms using the kindling method.

Other Effects of Brief Ethanol Exposure

There are a number of other effects of long-term alcohol consumption that may be demonstrated following very short exposures. Tolerance, for example, has been generally assumed to be due to long-term abuse of alcohol. It is well established that a dose of ethanol just sufficient to impair nondrinkers will have little effect on an alcoholic (Kalant et al, 1970). Recent research, however, has indicated that even a single dose of ethanol can produce tolerance (LeBlanc, Kalant, & Gibbins, 1975). The effects of a single injection of alcohol were assessed in rats at various times after intraperitoneal administration. Disruption of behaviour soon after the injection was markedly less than the disruptive effect at the same blood alcohol level a short period of time later.

Pronounced changes in REM sleep, cortical potentials evoked by somatosensory stimuli, and alcohol-induced nystagmus due to brief exposures of ethanol has been reported. Knowles, Laverty, and Kuechler (1968), for example, found that 3.5 oz of alcohol at bedtime was sufficient to inhibit REM sleep during the first half of the night and to enhance it during the second half in a normal healthy subject. Six oz produced a complete suppression of REM activity and during the nights following such complete REM suppression the incidence of REM was increased. Beglieter, Poyecz, and Yerre-Grubstein (1974) found that 10 hr after consuming 3.2 g/kg/day for four days there was a significant increase in the recovery cycle of somatosensory evoked responses. Aschan, Bergstedt, Goldberg, and Laurell (1956) reported that positional nystagmus can be induced in humans after the metabolism of even small amounts of alcohol.

The effects of a single injection are not restricted to the central nervous system where the aforementioned phenomena likely occur. For example, one very large dose of ethanol produces an accumulation of fatty acids in the liver which becomes more pronounced after prolonged ethanol intake (cf. Lieber, 1970). Ugarte and Valenzuela (1971) concluded that small doses of ethanol administered intravenously stimulate pancreatic secretion in a manner analagous to the way more dramatic increases are produced by chronic alcohol exposures.

Hangover

Khan, Jensen, and Drough (1973) included vomiting, loss of appetite, heartburn, lassitude, thirst, tremor of hands and limbs, palpitation, weakness of joints, respiratory difficulties, sleeplessness, dizziness, headache, fatigue, sweating, disturbance of balance, pallor, nystagmus, general malaise, mood disturbance with anxiety and depression as possible symptoms of hangover. Although these are the most widely experienced aftereffects of drinking, little research has been done on them. One major problem has been the failure to come up with an appropriate operational definition of hangover. Thus everything from a headache (Wolff, 1963) to delirium tremens (Karpman, 1957) has been included as a symptom of hangover. Another problem has been the lack of suitable methods of inducing hangovers. Paradoxically only 10 to 20 percent of the subjects in experiments on hangovers actually report them (Chapman, 1970). As a result of these problems there is a general lack of studies attempting to identify factors which influence the incidence and severity of hangover (cf. Wolff, 1963).

Several indirect lines of evidence suggest that at least some hangover effects may be a product of withdrawal-produced increases in seizure susceptibility. For example, the hangover is typically observed as an aftereffect of drinking (Karvinen, Miettinen, & Ahlman, 1962) and as such has a very similar time course to the increases in seizure susceptibility seen upon the withdrawal of alcohol. In addition the results of the present experiment show that increases in seizure susceptibility, like the hangover, may be produced following the first

brief exposure to ethanol. Moreover, additional alcohol is one of the best known remedies for both hangover and the relief of the severe withdrawal effects after long-term ethanol consumption (Wallgren & Barry, 1970). An additional piece of evidence suggesting that hangover is a mild withdrawal effect is the similarity of symptoms of hangover to those following termination of long-term alcohol consumption (Wallgren & Barry, 1970). In fact, there is little, if any, difference between subjective reports of a mild withdrawal syndrome and a severe hangover (cf. Victor & Wolfe, 1973).

Thus, there is evidence to suggest that at least some of the symptoms of hangover may be related to changes in seizure susceptibility. Despite its obvious importance, this relation has gone unnoticed. Many theories of the hangover have been put forward (Chapman, 1970) however with only one exception (Wallgren & Barry, 1970) none have considered hangovers to be related to changes in seizure susceptibility.

Failure to recognize the hangover as an early manifestation of withdrawal effects typically produced following long-term ethanol exposure is one of the main reasons that hangovers have attracted little experimental interest. This is unfortunate since the hangover may play an important role in the development of more severe withdrawal effects and may provide a valuable means to follow the genesis of these withdrawal effects.

Relation of Pathological Effects of Alcohol Exposure to Withdrawal Seizures

There is considerable indirect evidence that alcohol consumption

in humans is related to increases in seizure susceptibility. In this section the possibility that some of the effects of alcohol consumption may be a direct consequence of these convulsive effects is considered. Three hypotheses will be discussed: 1) that the serious withdrawal effects observed in chronic alcholics are at least partially the result of the repeated experience of the convulsive effects of short ethanol exposures; 2) that electrographic epileptic activity related to the withdrawal of alcohol may be a factor in alcohol-related blackouts; and 3) that electrographic epileptic activity may be responsible for intellectual deficits related to chronic alcohol consumption.

The first hypothesis concerns the possibility that repeated experience of the convulsive effects of brief ethanol exposures may play an important role in determining the severity of the alcohol withdrawal syndrome in cchronic alcoholics. It was pointed out earlier that there is a gradual increase in stimulus-induced epileptic activity when localized brain stimulation is repeatedly administered to the amygdala of various species. This was termed the kindling effect. The kindling effect has typically been associated with the progressive development of seizures in response to periodic, local brain stimulation, however, recent developments in this literature strongly suggest that the changes associated with brain stimulation may simply be specific manifestations of a phenomenon general to all convulsants. Kindling-like effects have been recorded after repeated administration of a variety of convulsive agents. For example, Mason and Cooper (1972) administered "subconvulsive" doses of pentylenetetrazol to rats at 3-day intervals. After a few

injections minor myoclonic responses developed and eventually grand mal seizures were elicited in some animals. Leech (1972) observed that mice which are initially resistant to audiogenic seizures eventually become responsive to the stimulation if they are exposed to a short burst of audio stimulation each day. At first the sound elicited little more than a startle response in most mice of "resistant" strains but after several days of exposure, fits of running and jumping were elicited. With a few additional stimulations grand mal convulsions were typically produced. Prichard, Gallagher, and Glaser (1969) found that if seizures were elicited with flurothyl ether at a rate of not more than once a day there was a progressive reduction of seizure threshold in rats, mice, and guinea pigs. With each presentation of flurothyl, there was a decline in the latency to the first myoclonic jerk and to sustained convulsion, and an increase in the severity of the MS pattern. Vosu and Wise (1975) discovered that carbachol injected daily into the amygdala, hippocampus, or caudate of rats at "subconvulsive" doses eventually elicited MSs. Finally, Ramer and Pinel (1974) found a gradual intensification of the seizures elicited by periodic electroconvulsive shock. In each of these situations, a specific level of the convulsive agent comes to elicit more severe seizures following the periodic presentation of the convulsant. Thus, because of the apparent generality of the kindling phenomenon, it appears possible that the epileptic consequences of alcohol exposure may also be intensified with repeated presentation and withdrawal.

There is little research providing direct support for this suggestion, however there are several observations consistent with it.

First are the observations by Walker and Zornetzer (1974) that withdrawal after a second ethanol exposure in rodents produces withdrawal effects more severe than those observed after the first. Second, Mendelson et al.(1966) and Isbell et al.(1955) found that alcoholics experiencing withdrawal effects following the cessation of drinking, are more likely to develop withdrawal effects following subsequent periods of drinking. A third relevant observation comes from the barbiturate literature. Wulff (1960) found that patients who had previously exhibited epileptic symptoms during barbiturate withdrawal had twice the incidence of withdrawal convulsions than those who had not previously experienced them.

Although these data are consistent with the contention that alcohol withdrawal convulsive effects intensify they are by no means conclusive. In all of these studies the number of withdrawal episodes and the duration of drug exposure are confounded. Thus, the aforementioned evidence is consistent with a number of equally plausible explanations. Nevertheless, the hypothesis deserves serious considerations because of the implications it has for the treatment and understanding the effects of alcohol consumption; a sudden temporary cessation of drinking would not only result in increases in seizure susceptibility, but would also increase the severity of subsequent withdrawal symptoms. Since the hangover may be an early manifestation of convulsive effects of alcohol withdrawal, it is an extremely important phenomenon with regard to the present discussion. The susceptibility and frequency of hangovers may play an important role in the gensis of more severe forms of alcohol-withdrawal-produced convulsive effects.

The second hypothesis suggests a relation between the electrographic epileptic activity associated with alcohol withdrawal and alcohol amnesia

or blackouts. Alcohol blackouts are usually defined as memory loss without loss of consciousness for events that occur during drinking (Lisman, 1974). It is well established that epileptiform electrographic discharges with no motor correlates can have amnesic effects in both humans (Browne, Penry, Porter, & Dreifuss, 1974) and in animals (Woodruff, 1974). Thus, it is not unreasonable to believe that some of the memory problems of alcoholics are related to subconvulsive epileptic activity triggered by alcohol withdrawal (Goodwin, Crane, & Guze, 1969a; Ryback, 1970).

There is some empirical evidence suggesting that blackouts are related to withdrawal-produced electrographic abnormalities. For example, Goodwin et al. (1969) found that the severity of blackouts in hospitalized alcoholics was positively related to the extent and duration of alcohol abuse and to a history of head trauma, two factors which have been shown to be related to the incidence of withdrawal-produced epileptiform abnormalities (cf. Victor, 1968). In addition, it is now well-known that both blackouts and seizure-related amnesia typically occur as losses of blocks of time (Goodwin et al. 1969a,b; Ryback, 1970).

The third hypothesis is that learning and intellectual deficits that sometimes occur for several years following the withdrawal from long-term alcohol exposure (Farmer, 1973; Kleinknecht & Goldstein, 1972; Page & Linden, 1974) may also be a result of epileptic activity present in the brain after the withdrawal of alcohol. This possibility has generally been overlooked since the gross behavioural manifestations of the underlying epileptiform discharges do not usually persist for more than a few days after withdrawal (cf. Victor, 1968). Thus, the withdrawal effects have been viewed as short-term events which cannot be responsible for the long-term cognitive

deficits frequently observed following chronic alcohol exposure. However, it is now strongly suggested that withdrawal-produced epileptiform abnormalities can persist for several months after alcohol withdrawal. Bennett (1960), for example, found abnormal spike and wave activity in many patients' EEG for up to several months after the alcohol withdrawal. Epileptiform EEG activity has also been found in rats for up to several months following the withdrawal from long-term alcohol exposure (Mucha & Pinel, unpublished observations).

There are two additional lines of evidence that support this hypothesis. First, the deficits in chronic alcoholics are similar to those seen in some patients with epileptiform activity induced by other agents (cf. Woodruff, 1974). A second line of evidence was provided by Bennett (1960) who noted that in many alcoholics with EEG abnormalities the recovery of the behavioural deficits paralleled recovery of the EEG.

Relevant for all three hypotheses is the study of subcortical EEG epileptiform activity during the withdrawal of alcohol. There is almost no research on this in humans; however, recent animal research indicates that subcortical, rather than cortical structures, play the primary role in the genesis of epileptic activity during the withdrawal period.

This finding is important since the hypotheses concern phenomena associated with subcortical structures. For example, the amygdala is the most responsive structure for kindling seizures (Goddard et al, 1969). Similarly, hippocampal and amygdaloid ADs with no MSs result in profound learning and retention deficits in cats (Kesner & Doty, 1968).

Thus, the study of alcohol-related epileptic effects has implications for the understanding of three additional areas of alcohol related

pathology. The evidence for the involvement of seizures in these phenomena remains without direct support primarily because of the difficulty in conducting the appropriate experiments on human patients. However, the formulation of these hypotheses was based on clearly established findings in the epilepsy literature.

Concept of Physical Dependence

An individual is said to be physically dependent on a drug when its withdrawal triggers an illness called a withdrawal syndrome, whose exact form depends to some extent on the nature of the drug. For example, the withdrawal of barbiturates and alcohol produces a syndrome with convulsions as one of the most prominent symptoms (Essig, 1972). Withdrawal of morphine, however, rarely results in convulsions (Seever & Deneau, 1963). Thus, the term "physical dependence" can be used to describe the unknown characteristics which differentiate a healthy subject from one in which a withdrawal syndrome can be easily triggered, and as such it serves a useful function.

Unfortunately, the concept of physical dependence has been of little scientific use because there is no way of measuring it other than in terms of the withdrawal syndrome (Seevers & Deneau, 1963). Some studies have noted that tolerance develops at approximately the same time as physical dependence (Isbell et al., 1955). However, the level of tolerance is not a good measure of physical dependence as defined by the severity of the withdrawal syndrome. The duration of alcohol exposure required to produce asymptotic withdrawal effects is considerably greater than that required to produce maximum tolerance (Kalant et al., 1970). Thus,

because physical dependence cannot be defined without withdrawing ethanol, its many studies in the literature are in fact studies of the alcohol withdrawal syndrome.

Although the term "physical dependence" is useful in some contexts, its use has had two unfortunate effects. First, individuals have used the term as if physical dependence had an existence separate from the withdrawal symptoms that define it, and, worse, they have used the term to explain the presence of the withdrawal symptoms. Secondly, the fact that the processes underlying the development of susceptibility to withdrawal effects are generally referred to by a single term has caused some investigators to lose sight of the fact that there is probably more than a single factor involved. Victor (1968), for example, described the appearance of two distinct groups of symptoms occurring at two different times after withdrawal from longterm intoxication. The group characterized by tremors, hallucinations and convulsions occurred between 6 and 48 hr after the withdrawal of alcohol. The other group of symptoms comprised of delirium and autonomic overactivity almost invariably came after the first group.

Thus, although the term 'physical dependence' is useful when used appropriately, its overall effect has been to complicate the study of the production of various withdrawal reactions. Nevertheless, the present results suggest that physical dependence, as defined by withdrawal-produced convulsive effects, begins to develop with an organism's first exposure to ethanol.

CONCLUSION

In the present experiment it was clearly established that an organism's first exposure to ethanol could have convulsive withdrawal effects. Following the metabolism of a single dose of ethanol, kindled seizures elicited by amygdaloid stimulation were longer than usual. Thus, pathological changes which underly withdrawal convulsions appear to be present to a minor degree even after the first exposure to ethanol.

The methods used to demonstrate these effects were very effective in following the timecourse of changes in seizure susceptibility in a single animal. They may prove valuable for similar purposes in other areas.

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APPENDICES

Appendix A

Analysis of Variance Tables for Experiment 1

Analysis of Variance Table for Experiment 1 AD Duration

Source	SS	df	MS	F
Between Subjects				
Drug	4015.5	1	4015.5	.90
Subj/groups	56184.1	13	4321.8	
Within Subjects				
Time	65341.0	17	3843.6	9.14*
Time x Drug	39490.8	17	2323.0	5.50*
Time x Subj/groups	92877.7	221	420.3	

* $p < .05$

Analysis of Variance Table for Experiment 1 MS Duration

Source	SS	df	MS	F
Between Subjects				
Drug	678.1	1	678.1	3.74
Subj/groups	2352.7	13	180.9	
Within Subjects				
Time	4189.3	17	246.4	10.50*
Time x Drug	5152.3	17	303.0	12.90*
Time x Subj/groups	5183.0	221	23.5	

* $p < .05$

Analysis of Variance Table for Simple Effects of Experiment 1 AD Duration

Source	SS	df	MS	F
Between Subjects				
Drug at T_1	168.3	1	168.3	.26
" T_2	146.7	1	146.7	.23
" T_3	531.2	1	531.2	.83
" T_4	11640.6	1	11640.6	18.27
" T_5	6862.9	1	6862.9	10.70
" T_6	1884.3	1	1884.3	2.95
" T_7	1845.2	1	1845.2	2.89
" T_8	936.6	1	936.6	1.47
" T_9	2099.4	1	2099.4	3.29
" T_{10}	27.6	1	27.6	.043
" T_{11}	21.2	1	21.2	.03
" T_{12}	976.0	1	976.0	1.53
" T_{13}	1788.5	1	1788.5	2.80
" T_{14}	9812.5	1	9812.5	15.40*
" T_{15}	891.5	1	891.5	1.39
" T_{16}	1666.0	1	1666.0	2.61
" T_{17}	318.3	1	318.3	.49
" T_{18}	1901.3	1	1901.3	2.98
Within cell	149061.7	234	637.0	
Within subjects				
Time at D_1	106487.0	17	6263.0	14.90**
" D_2	5114.0	17	300.0	.71
Time x Drug	39490.8	17	2323.0	5.50
Time x Subj/groups	92877.6	221	2420.0	

*$p < .05/18$
**$p < .05/2$

Analysis of Variance Table for Simple Effects of Experiment 1 MS Duration

Source	SS	df	MS	F
Between Subjects				
Drug at T_1	112.2	1	112.2	3.40
" T_2	63.8	1	63.8	1.98
" T_3	107.0	1	107.0	3.32
" T_4	3226.3	1	3226.3	100.10*
" T_5	1241.1	1	1241.1	38.54*
" T_6	711.9	1	711.9	22.10*
" T_7	28.3	1	28.3	.88
" T_8	224.5	1	224.5	6.90
" T_9	110.0	1	110.0	3.40
" T_{10}	23.5	1	23.5	.73
" T_{11}	3.5	1	3.5	.10
" T_{12}	4.5	1	4.5	.13
" T_{13}	16.8	1	16.8	.53
" T_{14}	19.1	1	19.1	.59
" T_{15}	6.5	1	6.5	.20
" T_{16}	4.5	1	4.5	.13
" T_{17}	19.4	1	19.4	.60
" T_{18}	22.0	1	22.0	.68
Within cell	7575.7	234	32.2	
Within subjects				
Time at D_1	8269.0	17	486.4	20.70**
" D_2	1522.2	17	89.5	3.60**
Time x Drug	5152.3	17	303.0	
Time x Subj/groups	5183.0	221	23.5	

*$p < .05/18$
**$p < .05/2$

Appendix B

Analysis of Variance Tables for Experiment 2

Analysis of Variance Table for Experiment 2 AD Duration

Source	SS	df	MS	F
Between Subjects				
Order	168.7	1	168.7	.11
Subj/groups	11980.2	8	1497.5	
Within Subjects				
Drug	11172.2	1	11172.2	18.05
Drug x Order	726.7	1	726.7	1.17
Drug x Subj/groups	4951.2	8	618.9	
Time	34247.1	11	3113.4	21.17
Time x Order	771.6	11	70.1	.04
Time x Subj/groups	12938.0	88	147.0	
Drug x Time	44609.9	11	4055.4	25.73
Drug x Time x Order	1884.4	11	171.3	1.08
Drug x Time x Subj/groups	13866.8	88	157.6	

Analysis of Variance Table for Experiment 2 MS Duration

Source	SS	df	MS	F
Between Subjects				
Order	1.4	1	1.4	.01
Subj/groups	1640.7	8	205.1	
Within Subjects				
Drug	946.6	1	946.6	32.89
Drug x Order	176.0	1	176.0	6.11
Drug x Subj/groups	230.2	8	28.8	
Time	13521.7	11	1229.2	35.97
Time x Order	396.3	11	36.0	1.05
Time x Subj/groups	3006.6	88	34.2	
Drug x Time	8069.0	11	733.6	28.89
Drug x Time x Order	208.5	11	19.0	0.74
Drug x Time x Subj/groups	2234.5	88	25.4	

Appendix C

Analysis of Variance Tables for Experiment 3

Analysis of Variance Table for Experiment 3 AD Duration

Source	SS	df	MS	F
Between Subjects				
Order 1	1092.5	1	1092.5	1.19
Order 2	709.4	3	236.5	0.25
Order 1 x Order 2	2623.3	3	874.4	0.95
Subj/groups	3644.4	4	911.1	
Drug	1121.3	1	1121.3	13.39
Drug x Order 1	431.9	1	431.9	5.15
Drug x Order 2	298.3	3	99.4	1.18
Drug x Order 1 x Order 2	3208.2	3	1069.4	12.77
Drug x Subj/groups	334.9	4	83.7	
Time	16918.3	3	5639.4	72.95
Time x Order 1	104.6	3	34.8	0.45
Time x Order 2	471.3	9	52.3	0.67
Time x Order 1 x Order 2	3943.9	9	104.8	1.35
Time x Subj/groups	927.6	12	77.3	
Drug x Time	18924.3	3	6308.1	46.80
Drug x Time x Order 1	427.5	3	142.5	1.05
Drug x Time x Order 2	1172.5	9	130.2	0.96
Drug x Time x Order 1 x Order 2	2502.0	9	278.0	2.06
Drug x Time x Subj/groups	1617.1	12	134.7	

Analysis of Variance Table for Experiment 3 MS Duration

Source	SS	df	MS	F
Between Subjects				
Order 1	33.7	1	33.7	0.29
Order 2	343.1	3	114.3	0.99
Order 1 x Order 2	327.9	3	109.3	0.94
Subj/groups	461.3	4	115.3	
Drug	2285.9	1	2285.9	254.32
Drug x Order 1	1.2	1	1.2	0.13
Drug x Order 2	134.9	3	44.9	5.00
Drug x Order 1 x Order 2	124.2	3	41.3	4.60
Drug x Subj/groups	35.9	4	8.9	
Time	4685.2	3	1561.7	73.82
Time x Order 1	194.1	3	64.6	3.05
Time x Order 2	102.9	9	11.4	0.54
Time x Order 1 x Order 2	192.1	9	21.3	1.00
Time x Subj/groups	253.9	12	21.1	
Drug x Time	6203.3	3	2067.7	50.97
Drug x Time x Order 1	6.9	3	2.3	0.05
Drug x Time x Order 2	233.9	9	25.9	0.64
Drug x Time x Order 1 x Order 2	387.9	9	43.0	1.06
Drug x Time x Subj/groups	486.7	12	40.5	

Appendix D

Analysis of Variance Tables for Experiment 4

Analysis of Variance Table for Experiment 4 AD Duration

Source	SS	df	MS	F
Order	6915.1	3	2305.0	2.07
Subj/groups	13327.6	12	1110.6	
Condition	2015.2	3	671.7	3.84
Condition x Order	2021.9	9	224.6	1.28
Condition x Subj/groups	6281.7	36	174.4	

Analysis of Variance Table for Experiment 4 MS Duration

Source	SS	df	MS	F
Order	435.5	3	145.1	1.05
Subj/groups	1656.5	12	138.0	
Condition	140.4	3	46.9	2.02
Condition x Order	259.5	9	28.8	1.24
Condition x Subj/groups	833.7	36	23.1	

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