INTENSIFICATION OF THE ALCOHOL WITHDRAWAL SYNDROME BY ANTECEDENT ELECTROCONVULSIVE SHOCKS

by

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Abstract

When periodic electroconvulsive shocks (ECSs) were administered, a progressive intensification of the motor seizure (MS) pattern occurred. This effect was observed when ECSs at either 15 or 75 mA were administered at 3-day, but not 1-hr intervals. The magnitude of the increase in severity of the MS pattern was a function of the number of ECSs which approached asymptote, in these experiments, at approximately ten ECSs. Periodic ECSs were also found to potentiate the alcohol withdrawal syndrome. In general, those conditions which were found to facilitate the kindling of MSs were the same as those which produced the potentiation of the alcohol withdrawal syndrome. Furthermore, this potentiation was found to persist up to 3 weeks after the last ECS under the conditions used in these experiments. Finally, the potentiation of the alcohol withdrawal syndrome occurred even after the MSs had been pharmacologically blocked. The results of these experiments were discussed in light of their implications to both basic and clinical research.

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Introduction

When electroconvulsive shocks (ECSs) are periodically administered to experimental animals, the motor seizures (MSs) elicited by each successive ECS grow progressively more severe. The present investigations demonstrated that a series of ECSs also intensify an organism's convulsive response to subsequent alcohol withdrawal, and they provided information on the effects of various parametric manipulations on this interaction.

The idea that a subject's response to a convulsive treatment might be intensified by prior, repeated ECSs evolved from research on the so-called kindling effect. Kindling is the development and intensification of stimulation-induced seizures produced by periodic, electrical stimulation of discrete areas of the brain at intensities which initially elicit no observable responses. Thus, a brief review of some of the more relevant aspects of the kindling effect comprises the first section of the Introduction. The second section includes reports of kindling-like effects produced by the periodic administration of agents other than local, electrical brain stimulation and the third includes reports that kindling with one agent can intensify an organism's response to other convulsive agents. The fourth and final section of the Introduction contains a summary of the rationale and objective of the present series of investigations.

Kindling

Definition of kindling. Periodic, electrical stimulation of any of a number of sites throughout the olfactory-limbic system or cortex, even at intensities initially too low to produce any manifest behavioural or electrographic effects, can lead to the elicitation and gradual intensification of MSs (Goddard, McIntyre, & Leech, 1969; Racine, 1975). For example, if the rat amygdaloid complex is stimulated once per day at a level which initially produces no observable effects, eventually the afterdischarge (AD) threshold may be reduced to the point that subsequent stimulations reliably elicit focal ADs (Racine, 1972a). Each time a focal AD is elicited, it propagates to progressively more areas of the brain, and becomes more complex in waveform (Racine, 1972b). It appears to be this generalization of the AD which is correlated with the development and intensification of clonic MSs (Racine, 1972b). Although kindling can be produced from any one of a variety of brain sites, since Goddard et al. (1969) found that the amygdala required the fewest number of stimulations to produce MSs, the majority of investigations of kindling have employed amygdaloid stimulations.

The exact form of kindling is a function of the site of stimulation. During the course of kindling from most subcortical sites, even at high intensities, the first few stimulations produce no obvious behavioural effects (Racine, 1972b), but after several stimulations, mild facial clonus is regularly elicited. Then, with each successive stimulation, the clonus becomes progressively more general until eventually each

stimulation reliably elicites a clonic MS characterized by a progression of symptoms through facial and forelimb clonus, rearing and culminating with a loss of equilibrium. In contrast, the first stimulation of the anterior neocortex, for example, typically elicits clonus of the face or forelimbs similar to that seen during the early stages of kindling with subcortical stimulations. These neocortical MSs grow progressively more intense with subsequent stimulations until each stimulation reliably elicits a clonic MS characterized by an immediate loss of equilibrium and culminating in a mild form of tonic extension (Racine, 1975).

Since its original description in rats by Goddard (1967), kindling has been reported in mice (Leech, 1972), cats (Morrell, 1973), rabbits (Tanaka, 1972), frogs (Morrell & Tsuru, 1975), and primates (Wada & Sato, 1973). There has even been some indication that kindling can occur in humans (Ervin, Mark, & Stevens, 1969; Stevens, Mark, Ervin, Pacheco, & Suematsu, 1969). Although the exact motor manifestations of kindling are somewhat different in various species, a progressive pattern of development and intensification of MSs in response to the stimulations has been reported to be similar in each species.

Characteristics of kindling. One of the more striking features of kindling is that the changes in neural function which underly it are relatively enduring. Goddard et al. (1969) rekindled rats after a 12-week, stimulation-free period and found a savings of about 90% in the number of stimulations required to elicit generalized MSs. Racine (1972a) found that reductions in the AD threshold were also relatively

enduring, lasting at least 6 weeks. Although the permanence of the changes in MSs is more striking and has thus been studied in more detail, the changes in AD threshold are particularly important since they can be produced by subthreshold stimulations (Pinel, Skelton, & Mucha, 1975). Thus, periodic stimulations can produce lasting changes in the organism even when neither ADs nor MSs are elicited.

Another striking feature of kindling is the eventual development of spontaneous MSs. Recent experiments have indicated that amygdaloid kindling can eventually produce spontaneously recurring MSs in cats (Wada & Sato, 1973; Wada, Sato, & Corcoran, 1974), rats (Pinel, Mucha, & Phillips, 1975; Rovner & Pinel, 1976) and baboons (Wada, Osawa, & Mizoguichi, 1975). Although suggestions have been made that kindling could be a model of epileptogenesis, until these studies were reported this suggestion had to be questioned. The primary characteristic of epilepsy is that MSs occur spontaneously whereas with the early studies of kindling, MSs had to be elicited. These more recent studies have thus established that kindling can lead to the progressive development of a bona fide epileptic syndrome. Thus, the kindling model could prove to be a valuable tool in the controlled study of the factors associated with epileptogenesis.

Factors influencing kindling. The duration of the interval between stimulations is an important determinant of the number of stimulations to the first generalized MS. Goddard et al. (1969) were the first to report an inverse relation between the number of stimulations

to the first generalized MS and the duration of the interstimulation intervals. They were unable to kindle animals with intervals of less than 20 min; whereas, the fewest number of stimulations were found to be required at intervals of 24 hr or more. As a result, stimulations have been typically administered at daily intervals in most kindling studies. Racine, Gartner, Burnham, and Levitan (1973) confirmed the general inverse relation between the interstimulation interval and kindling rate and showed that massed stimulations neither produced kindling nor significantly reduced the number of stimulations required for subsequent kindling at longer intervals. However, Racine et al. (1973) argued that animals could be efficiently kindled at intervals as short as 11 hr.

Current intensity also appears to be an important factor in determining the number of stimulations required for kindling; higher intensities generally produce kindling in fewer stimulations. This relation, however, seems to be primarily attributable to the fact that current intensities too low to elicit ADs do not kindle MSs. Racine (1972b) found that no kindling occurred in response to stimulations kept below the AD threshold. This is not surprising in that Racine established, in the same experiment, that it was the spread of ADs which was correlated with MS development. In this study, Racine found no differences in the number of stimulations to the first generalized MS elicited by two, suprathreshold-intensity stimulations. He, therefore, concluded that the entire effect of current intensity depended on whether or not ADs were elicited by the stimulations (Racine, 1972b). Pinel, Phillips,

and Deol (1974), however, did find some differences in kindling with different suprathreshold intensities. When two groups of rats were kindled at two intensities, one just above threshold ($\bar{X}$ = 75 μA) and the other at a much higher level ($\bar{X}$ = 500 μA), the duration of the ADs and MSs elicited by the higher intensity stimulations varied less between stimulations. There was also a slight, but significantly more rapid progression of kindling at the higher intensities.

Generality of Kindling: Kindling with Other Agents

Although the term "kindling" is typically used in reference to the development and intensification of MSs produced by local, electrical brain stimulations, similar effects have been reported following periodic administrations of a variety of other potentially convulsive agents.

Metrazol. Mason and Cooper (1972) administered metrazol (pentylenetetrazol) to rats at 3-day intervals, in i.p. doses which were initially subconvulsive. After several injections, mild convulsive responses were elicited which became progressively more intense with each injection until generalized clonic MSs, sometimes culminating in myotonus, were reliably produced. When animals previously kindled with metrazol were retested after a 21-day injection-free period, no diminution in the severity of the convulsive response to metrazol was noted. Thus, at least superficially, kindling with metrazol seems to be like

kindling with local electrical brain stimulation.

Fluorothyl. With each successive periodic exposure to fluorothyl vapour (hexafluorodiethyl ether) decreases in the latency to sustained MS and increases in the severity of the overall MS pattern may result (Prichard, Gallagher, & Glaser, 1968). Fluorothyl is a volatile convulsive ether typically administered by inhalation, and produces MSs which closely resemble those produced by metrazol. Prichard et al. (1968) found that progressive increases in the severity of the fluorothyl-induced MSs were produced only when the ether was presented at intervals of at least 24 hr. When fluorothyl was presented at 1-hr intervals, the latency to sustained MS was temporarily increased. MSs elicited at intervals of 20 min or less by local brain stimulation have been found to produce similar inhibitory effects (Goddard et al., 1969). Thus, although fluorothyl and local brain stimulation are similar in their response to the effects of interval, the critical intervals appear to be quite different. Whereas kindling will proceed with local brain stimulations administered once every hr, fluorothyl presented at this interval produces marked inhibitory effects.

Carbachol. Vosu and Wise (1975) compared the results of direct injections of carbachol, a cholinomimetic, into the amygdala, caudate, and hippocampus once every other day. They found a progressive development of MSs at all sites comparable to that observed by Goddard et al. (1969) with local, electrical stimulation of these same areas. More-

over, the relative kindling rates at the three sites were similar to the relative rates of kindling with electrical stimulation of the same sites (Goddard et al., 1969); the amygdala required fewer injections than did the caudate, and the caudate fewer than the hippocampus in both experiments.

Local anesthetics. Recently the development and intensification of MSs was reported with periodic administrations of two forms of local anesthetic, cocaine (Post & Kopanda, 1975; Post, Kopanda, & Black, 1975) and lidocaine (Post, Kopanda, & Lee, 1975). For example, when initially subconvulsive i.p. doses of lidocaine were administered to rats once every day, MSs which closely resembled the generalized clonic MSs elicited by amygdaloid stimulations were eventually elicited. A similar development of epileptogenicity was produced by periodic i.v. injections of cocaine in rhesus monkeys (Post, Kopanda, & Black, 1975).

Audiogenic stimulation. Leech (1972) observed that mice which were initially resistant to audiogenically-induced seizures eventually became responsive if they were exposed to short bursts of high-intensity audiogenic stimulation (noise) once each day. At first the sound elicited no more than a startle response in most of the mice of the "resistant" strains, but after a few exposures, paroxysms of running and jumping were elicited. MSs could be eventually elicited in this manner in even the most "resistant" strains.

Electroconvulsive shock. From the examples previously discussed, it is clear that periodic administrations of a variety of agents can produce kindling-like effects. The effects of repeated ECSs, however, stand in apparent contradiction to the other kindling-like effects discussed in this Introduction. In both humans (Holmberg, 1954) and experimental animals (Essig & Flanary, 1966; Essig, Groce, & Williamson, 1961) an increase, rather than a decrease, in the convulsive threshold had been reported as the usual result of periodic ECSs. For example, Essig et al. (1961) found that if ECSs were administered to cats twice a day, a progressive increase in the convulsive threshold was produced which returned to normal levels a few days after the stimulations were discontinued. Even greater increases in threshold were produced when ECSs were administered four times a day. Similarly, Holmberg (1954) found that in order to produce successive convulsions of comparable severity in human patients, the current intensity had to be progressively increased.

Ramer and Pinel (1975) examined the apparent discrepancy between the effects of repeated ECSs and those of other convulsive agents. In one of their studies, ECSs were administered once every three days to two groups of rats; the subjects in one group were stimulated at 15 mA and the other at 25. At both intensities the results were the same; with each successive stimulation there was a progressive increase in the severity of the MS pattern as reflected by shorter latencies to fore- and hindlimb tonic extension and a greater extent of tonic extension. Thus, in contrast to the earlier reports, periodic ECSs pro-

duced kindling-like effects.

Ramer and Pinel (1975) postulated that this apparent contradiction between the results of previous experiments and their own could be accounted for by differences in the interval between ECS administrations. To test this hypothesis, ten ECSs were administered to different groups of rats at 1-hr, 1-day, or 3-day intervals. In the 3-day condition a progressive increase in MS severity was produced, but in the 1-hr condition the results were consonant with the earlier reports; there was a gradual decrease in the severity of the MSs with each successive ECS. No systematic changes in MS severity were observed at the intermediate, 1-day, interval. A close examination of the earlier literature revealed that in those studies in which inhibition had been reported, intervals of less than 1 day had been used. However, kindling-like effects had been reported in those few earlier studies which had employed longer inter-ECS intervals (Zarrow, Pawlowski, & Denenberg, 1962; Pollack, Rosenthal, & Macey, 1963). Thus, the progressive effects of periodic ECSs are similar, at least superficially, to those produced by other kindling agents. Although the effects of the interval seem to be a function of the specific agent used, in general kindling occurs at long intervals; whereas, inhibitory effects are more prominent when the intervals are short.

Transfer of Kindling: Interactions between Agents

Periodic administrations of one kindling agent can greatly potentiate the animal's convulsive response to administrations of an-

other agent. For example, Pinel, Van Oot, and Mucha (1975) found that the alcohol withdrawal syndrome in rats was markedly intensified by 45 periodic, electrical stimulations of the amygdala. Those animals receiving suprathreshold stimulations (1 sec, 400-μA, 60-Hz) were kindled during the course of the experiment; whereas, those animals whose levels of stimulation were individually adjusted to keep them below the rapidly declining AD thresholds displayed no MSs. In both cases, however, subsequent alcohol withdrawal reactions were intensified. Thus, even stimulations at intensities too low to produce any obvious behavioural effects are capable of intensifying the alcohol withdrawal syndrome. The alcohol withdrawal syndrome has also been intensified by prior metrazol kindling (Pinel & Van Oot, 1975). Furthermore, Pinel, Skelton, and Mucha (1974) found that prior amygdaloid stimulations could intensify metrazol-induced MSs, whether or not MSs were produced by the amygdaloid stimulations themselves. However, in none of these studies was there a systematic relation between the degree of initial MS development in response to the repeated stimulations and the subsequent intensification of the reaction to the convulsive agents. For example, Pinel, Skelton, and Mucha (1975) attempted to relate aspects of an animal's responsiveness to amygdaloid stimulations to its subsequent responsiveness to metrazol-induced MSs. No clear relations between the number of ADs or MSs elicited by the stimulations; the initial or final AD threshold values, or the difference between them; or the number of ADs to the first generalized MS were found. Thus, although this transfer of susceptibility seems to be a reliable and reasonably general effect, the mechanisms underlying transfer are unclear. Although the

rationale for the present investigations was developed from the kindling literature, it appears that the kindling of MSs per se may not be essential for the potentiation of an animal's response to other agents.

Purpose

The initial purpose of the present investigations was to replicate the "ECS-kindling" effect, and then to investigate aspects of the interaction of periodic ECSs with the alcohol withdrawal syndrome. ECS-kindling, instead of kindling with some other agent, was studied for two major reasons. The first was that almost all previous kindling experiments have used amygdaloid stimulations, and a thorough investigation of kindling with some other agent might provide much-needed perspective to the kindling literature. Attempts to explain the basis of the kindling phenomenon have, thus far, been attempts to explain the basis of kindling with local brain stimulations. Kindling, however, seems to be a much more general phenomenon, and by studying its various forms, valuable insights into the mechanisms underlying it might be gained. The second reason was that because ECS is one of the most widely-used forms of clinical treatment, an investigation of kindling with ECS is likely to have practical implications. The interaction between periodic ECSs and the alcohol withdrawal syndrome was investigated also for largely practical reasons. Because of the extensive consumption of alcohol in our society, information concerning

alcohol withdrawal might be of greater practical relevance than comparable information on one of the less commonly occurring convulsive agents.

Experiment 1 confirmed the fact that periodic ECSs produce kindling-like effects, and also showed that ECSs can potentiate a subsequent alcohol withdrawal syndrome. In the remaining four experiments, various parametric influences on ECS-kindling and the interaction between periodic ECSs and the alcohol withdrawal syndrome were investigated. The intent of these experiments was not so much to find out what happens in a clinical situation, but rather to provide guidelines for future research and experimentation with actual clinical populations.

General Methods

The same basic methods were employed in each of the five experiments presented in this thesis. Thus, in this section, a description of the methodological features common to each experiment is presented. In each experiment, the progressive changes in the MSs produced by a series of ECSs and the effects of a series of ECSs on the incidence of withdrawal symptoms following a period of forced alcohol administrations was assessed.

Subjects

The subjects in each of the five experiments were 240- to 340-gm, male, black-hooded rats purchased from the Canadian Breeding Farms and Laboratories (La Prairie, Que.). Each animal was individually housed in a wire-mesh cage (24x18x18 cm) with ad libitum access to water and Purina Lab Chow pellets. The weight and general appearance of each animal were closely monitored, and if an animal lost weight, or appeared ill, that animal received supplements of wet, mashed food and/or penicillin until normal health was regained. Subjects were sacrificed by exposure to carbon dioxide following the University Federation of Animal Welfare standards of euthanasia (T-W-Fiennes, Harrison, Ray, & Scott, 1972).

Apparatus

Apparatus

ECSs were delivered from an 800-V, constant-current power source. Initiation of the ECS activated a timer which was videotaped with each MS so that the latency and duration of each phase of the MS could be accurately determined (± 0.05 sec).

The alcohol was administered intragastrically from a syringe through a #8, single-holed, rubber catheter. Following the withdrawal of alcohol, individual animals were observed in a transparent, Plexiglas box (20x16x40 cm) and the incidence of behavioural withdrawal symptoms was recorded.

Procedures

Surgery. Each animal was anesthetized with a combination of Nembutal (50 mg/cc; 50/mg/kg) and chloral hydrate (100 mg/cc; 100 mg/kg). The cranium was then exposed and four stainless steel skull screws were inserted, one in the centre of each of the two naso-frontalis bones, approximately 2 mm anterior to bregma, and the remaining two, 4 mm bilateral to the midline, midway between bregma and lambda. A 1.3-cm length of wire rod was then attached to each of the two posterior skull screws. The entire assembly was held in place and insulated from the surrounding tissue by a layer of dental acrylic from which protruded the two wire rods. Penicillin (30,000 I.U.) was routinely administered following surgery and each animal was handled

once every 2 days during the 1-week, post-operative recovery period.

ECS administrations. The 0.2-sec ECSs were delivered through a pair of alligator clips attached to the protruding wire rods. ECS typically elicits a MS characterized by an initial tonic flexion of the body followed by tonic extension and terminating in a brief period of clonus. During the development of tonic extension a "wave" of rigidity spreads caudally along the body until the back is arched and the forelimbs and the hindlimbs are fully extended. A MS elicited by low-intensity ECS, however, may lack this tonic phase completely or be characterized by a partial tonus limited to the rostral portions of the body (Woodbury, 1969). As a result of this relation between the current intensity and the caudal spread of tonic extension, the extent of tonic extension has been the most widely-used measure of MS severity (Pinel & Jones, 1973; Woodbury & Davenport, 1952). In the present experiments a score of 0 was assigned if no forelimb tonic extension occurred, 1 if there was forelimb, but no hindlimb extension, 2 if hindlimb extension was only partial, and 3 if there was complete hindlimb extension with both legs extending in a caudal direction.

Two other measures of MS severity employed in the present experiments, the latencies to fore- and hindlimb tonic extension, have also been used in previous experiments (Ramer & Pinel, 1975). These latencies were timed from the stimulus onset to the point when the limbs reached complete caudal extension. A fourth measure, the duration of forelimb tonus, was not used in previous experiments, but during

the course of preliminary experimentation, this measure was found to be both reliable and highly correlated with the other three measures. The duration of forelimb tonus was timed from the point at which full forelimb extension was reached to the first jerk of the forelimbs during the ensuing clonic phase.

In each of the five experiments an average of three animals suffered from ECS-induced paraplegia. These animals were immediately sacrificed and their data were not included in any of the analyses.

Alcohol administration. In each experiment those rats receiving alcohol were initially intubated with a relatively low, standard dose of 1000 mg/kg of a 20% ethanol and water solution (v/v). These subjects were injected a total of 42 times, once every 8 hr for 14 days. For the first 3 days, the level of intoxication was estimated 1 hr after each administration. Thereafter, the level of intoxication was assessed after only the second administration of each day. This particular method of inducing physical dependence to alcohol has been reported previously (Mucha, Pinel, & Van Oot, 1975), and similar methods have been employed by others (Majchrowicz, 1973).

The level of intoxication was rated for each animal on the basis of three behavioural measures; 1) homecage activity, 2) ability to manoeuvre on a vertical wire-mesh cliff, and 3) gait. The first measure was an assessment of the activity level of the undisturbed animal. Thus, an animal received a score of 0 if he was unconscious, 1 if conscious with his head resting on the floor, 2 if sitting still with his head

held up, or 3 if he was moving about the cage. Each subject was then removed from his cage and placed approximately 15 cm away from the top edge of a vertical wire-mesh cliff, 64 cm high and 24 cm wide. If the animal failed to take hold of the cliff, he received a score of 0; if he held, but then fell attempting to move, a score of 1; if he held and managed to climb down, he received a score of 2; or if he held and climbed up, a score of 3. Finally, each animal was placed on a flat, wooden surface and received a score of 0 for not moving, 1 for staggering and falling, 2 for walking very slowly, but steadily, or 3 for walking normally.

If an animal received more than one score of 2 or 3 on any of these three measures, subsequent doses of alcohol were increased by 200 mg/kg, but if the animal was judged to be intoxicated, the dose remained unchanged for subsequent administrations until there was evidence of tolerance. The first intoxicating doses served as measures of initial tolerance and the last intoxicating doses served as measures of the final tolerance of the animals to alcohol. The difference between these doses for each animal served as a convenient measure of the change in tolerance.

Because of the tendency of some animals to lose weight when chronically exposed to alcohol on this regimen (Mucha et al., 1976) all subjects were weighed daily. If any subject lost more than 10 gm of his pre-alcohol weight, alcohol was subsequently administered in solution with Metrecal rather than water until normal weight was regained. In this way the weights of all of the subjects were held fairly

constant throughout the five experiments.

Control animals not receiving alcohol were intubated with a 0.9% saline solution in volumes approximating the average amount of alcohol given to the experimental subjects on a particular day.

Withdrawal observation. Commencing 9 hr after the last alcohol administration, each animal was observed every 4 hr for ten consecutive 1-min periods. For each period, an experimenter unaware of the animal's experimental history, recorded the presence or absence of each of the following six withdrawal symptoms; 1) hyperreactivity to handling, 2) ear or eye twitching, 3) jaw clonus, 4) facial clonus, 5) head nodding, and/or 6) body jerks. Since there were five, 10-min observation sessions in all, the maximum score an animal could receive for each symptom was 50. These six measures have been found to reliably differentiate between animals undergoing even very mild withdrawal and those which are not experiencing withdrawal (Mucha et al., 1975). Each animal's score for a particular symptom was converted to a percentage of the control group's mean score for that symptom. Thus, the mean of the control animals on each symptom was 100%. For the purposes of statistical comparison the sum of these individual symptoms was taken and this "combined" withdrawal score was used for each animal. All computations were made relative to the group of animals which received only handling and alcohol. In each of the following experiments, the animals in this group will be referred to as the controls.

Statistical analysis. One-way analyses of variance were performed on the data obtained for the combined withdrawal scores, the three measures of alcohol tolerance, and three of the four measures of the ECS-induced MS severity. The data from the fourth measure of MS severity (degree of overall tonic extension) were ordinal, and thus required non-parametric analysis. In this case, Sign Tests and Mann-Whitney U tests were used to determine the significance of differences within and between groups respectively.

Because the number of groups in each experiment was greater than two, it was necessary to obtain an overall F value for the alcohol tolerance measures and the withdrawal scores. If this overall F was significant, subsequent individual comparisons were made with the Scheffé test. Because of the stringency of this test, these individual assessments were assumed to be significant at an alpha level of 0.10 as suggested by Scheffe (1959). For all other comparisons, however, the significance level was 0.05.

Experiment 1: ECS-Induced Intensification of the Alcohol Withdrawal Syndrome

Introduction

Experiment 1 had two major purposes. The first was to confirm and extend the initial report of ECS-kindling (Ramer & Pinel, 1975). Because of the widespread clinical use of ECS, a more detailed investigation of the possibility of kindling with periodic ECSs was warranted. The second purpose of Experiment 1 was to determine whether or not the changes induced by periodic ECSs could potentiate the convulsive symptoms produced by subsequent alcohol withdrawal. Both ECS-kindling and its potentiation of the alcohol withdrawal syndrome were studied at two different current intensities.

Methods

Following post-surgical recovery, 33 of the 45 subjects received the first 15-mA test ECS and were divided into three equal groups on the basis of their response to it. The subjects in two of these groups then received a series of eight treatment ECSs, one every 3 days at either 15 (n = 11), or 75 mA (n = 13). The animals in the third group (n = 9) received eight pseudo-ECSs; once every 3 days the electrodes were attached, but no current was passed. The animals in all three groups received the second 15-mA test ECS, 3 days after the eighth

treatment "stimulation". The 12 remaining animals were controls which did not receive either of the two test ECSs or any of the treatment ECSs. Thus, the animals in each of the two experimental groups received a total of ten ECSs; those in the pseudo-ECS group received two; and the control animals, none.

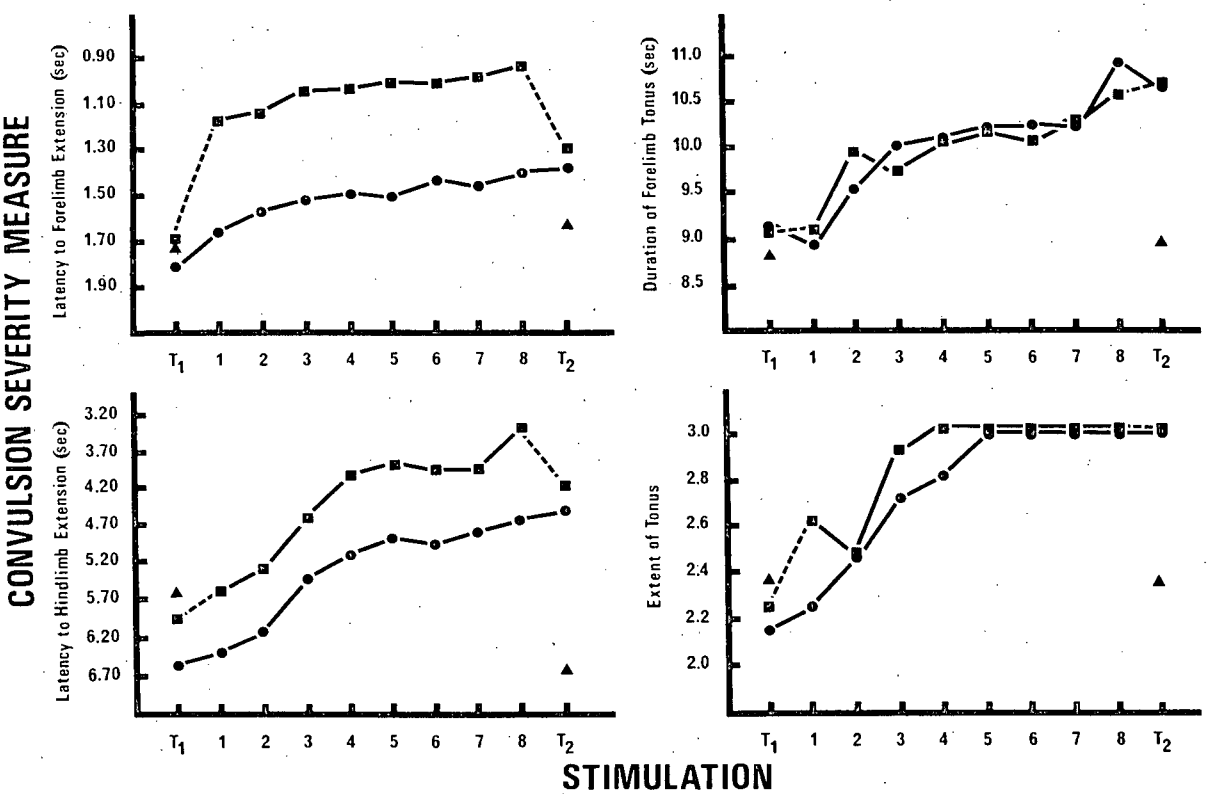
Beginning the day after the second test ECS, all 45 animals were subjected to the standard series of alcohol intubations. After the final intubation of the series, the incidence of overt withdrawal symptoms was assessed in each animal as previously described.

Results

At both intensities, periodic ECSs produced a kindling-like effect; with each successive stimulation, the elicited MSs became progressively more severe. Moreover, these ECSs were found to potentiate the alcohol withdrawal syndrome.

ECS-induced MSs. The progressive increase in the severity of the MSs elicited by the periodic ECSs was illustrated, at both intensities, by significant changes in all four measures of seizure severity (Figure 1). In each case, the mean response to the second test ECS was significantly more severe than the mean response to the first. In response to the second test stimulation, the subjects in both the 15- and 75-mA groups had significantly shorter latencies to forelimb (15 mA, F (1,20) = 12.59, $p < 0.01$; 75 mA, F (1,24) = 8.81, $p < 0.01$) and hindlimb

Figure 1. The progressive increase in MS severity produced by periodic ECSs. Animals in the experimental groups received two 15-mA test ECSs (T_1 & T_2) and eight treatment ECSs at either 15 (circles), or 75 mA (squares), presented at 3-day intervals. Animals in a pseudo-ECS group (triangles) received only the two 15-mA test ECSs.


CONVULSION SEVERITY MEASURE
Latency to Forelimb Extension (sec)
0.90
1.10
1.30
1.50
1.70
1.90
Duration of Forelimb Tonus (sec)
11.0
10.5
10.0
9.5
9.0
8.5
Latency to Hindlimb Extension (sec)
3.20
3.70
4.20
4.70
5.20
5.70
6.20
6.70
Extent of Tonus
3.0
2.8
2.6
2.4
2.2
2.0
T1 1 2 3 4 5 6 7 8 T2
STIMULATION

tonic extension (15 mA, F (1,20) = 6.68, $p < 0.05$; 75 mA, F (1,24) = 5.30, $p < 0.05$), significantly longer forelimb tonus (15 mA, F (1,20) = 8.45 $p < 0.01$; 75 mA, F (1,24) = 6.26, $p < 0.05$), and a significantly greater extent of tonic extension (15 mA, Sign Test, N = 5, x = 0, $p < 0.05$; 75 mA, Sign Test, N = 5, x = 0, $p < 0.05$). In contrast, the responses of the pseudo-ECS animals to the second test ECS were not significantly different than their responses to the first (all p's > 0.05).

The progressive increase in the mean severity of the convulsive response was also evident in the pattern of differences between the three treatment groups. Since these three groups had been equated on the basis of their responses to the initial 15-mA test ECS, there were no significant differences in their mean response to this ECS, (all p's > 0.05); however, the MSs elicited by the second 15-mA test ECS in both the 15- and 75-mA subjects were significantly more severe than those produced in the pseudo-ECS animals. There were significantly shorter latencies to forelimb (15 mA, F (1,18), = 4.92, $p < 0.05$; 75 mA, F (1,20) = 6.26, $p < 0.05$) and hindlimb tonic extension (15 mA, F (1,18) = 5.74, $p < 0.05$; 75 mA, F (1,20) = 6.26, $p < 0.05$),and longer forelimb tonus (15 mA, F (9,18) = 20.38, $p < 0.01$; 75 mA, F (1,20) = 9.76, $p < 0.01$). However, there were no significant differences between the animals of any of the three groups in extent of tonic extension (all p's > 0.05) induced by the second test ECS. Moreover, there were no significant differences between the mean responses of the 15- and 75-mA subjects to the second test ECS on any of the four measures of seizure severity (all p's > 0.05).

ECS-kindling was also illustrated by the progressive increase in the intensity of the responses to the eight treatment ECSs. The MSs elicited in both the 15- and 75-mA groups by the last treatment ECS were significantly more severe than those elicited by the first on each of the four measures of seizure severity (all p's < 0.05). During this phase of the experiment, the 75-mA ECSs consistently elicited mean responses more severe than those displayed by the 15-mA subjects. However, only the difference in the latency to forelimb tonic extension was significant (F (1,22) = 24.97, $p < 0.001$).

Alcohol tolerance. The animals which had previously been exposed to periodic ECSs generally tended to require higher doses of alcohol to initially induce and subsequently maintain intoxication throughout the alcohol exposure period (see Appendix I). Overall analyses of variance revealed significant differences among the four groups in initial tolerance (F (3,41) = 8.02, $p < 0.01$), final tolerance (F (3,41) = 6.43, $p < 0.01$), and the change in tolerance (F (3,41) = 4.98, $p < 0.01$). Although there were significant differences between certain groups on each of these measures, these differences appeared to be largely non-systematic and since they were not replicated in the remaining experiments, they were not presented here in detail (see Appendix II). These differences will be discussed in a more general context later in the thesis.

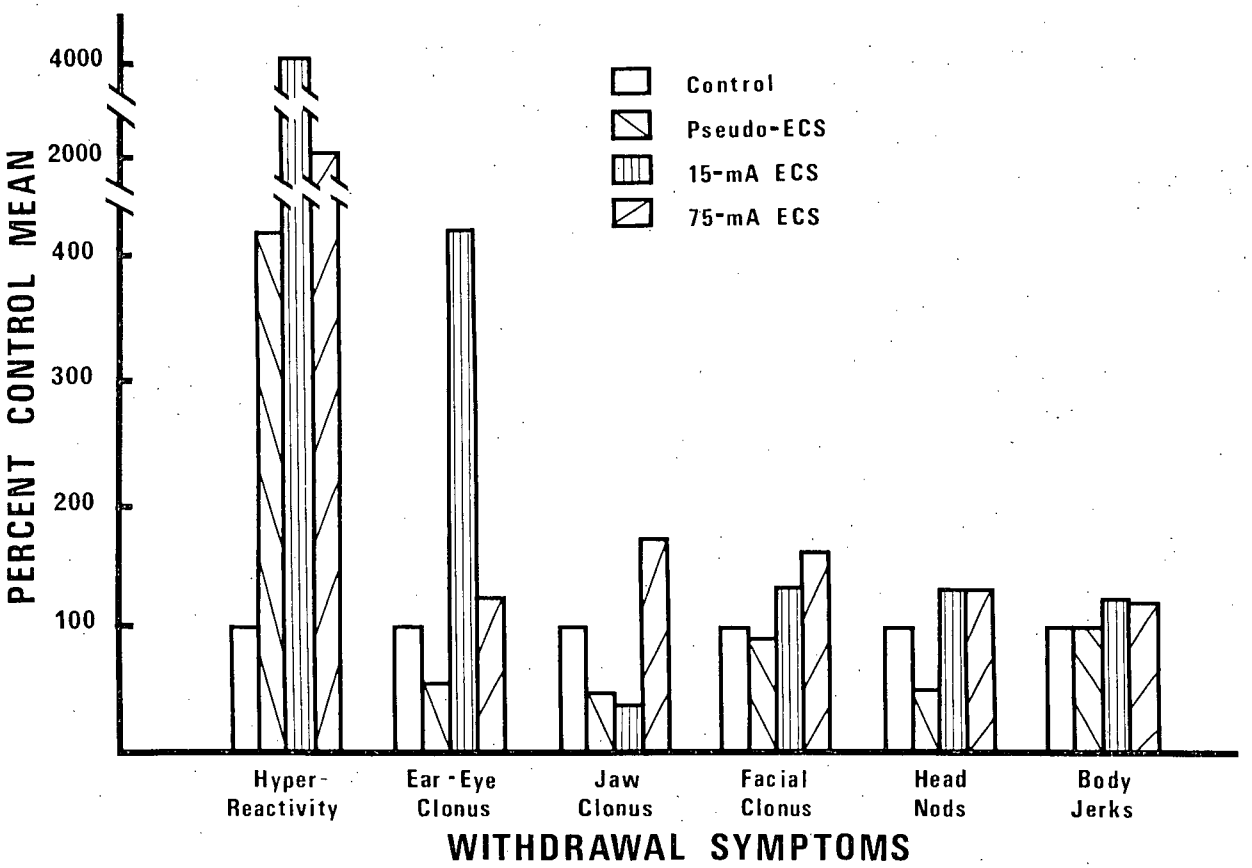
Withdrawal syndrome. Periodic ECSs at both 15-and 75 mA potentiated

the alcohol withdrawal syndrome (Figure 2). An overall analysis of variance revealed a significant difference among the four groups in the combined withdrawal scores (F (3,41) = 3.09, $p < 0.05$). Subsequent individual comparisons attributed this difference exclusively to the significantly less severe withdrawal responses of the animals in the control group than those of the animals in the 15-mA (F (1,21) = 6.55, $p < 0.10$) and the 75-mA, ECS groups (F (1,23) = 6.78, $p < 0.10$). None of the differences in the severity of the withdrawal syndrome between the control and pseudo-ECS groups, the 15- and 75-mA groups, or between the pseudo-ECS and either the 15- or 75-mA groups were significant (all p's > 0.10).

Discussion

The results of Experiment 1 confirm and extend the initial report of ECS-kindling by Ramer and Pinel (1975). In the Ramer and Pinel study, kindling occurred in response to periodic ECSs at either 15 or 25 mA. At both intensities latencies to fore- and hindlimb tonic extension became progressively shorter, and the extent of tonic extension increased. In Experiment 1, these same patterns of changes were observed. The results of Experiment 1 extend the findings of Ramer and Pinel (1975) in three ways. Firstly, the progressive increase in MS severity was also reflected in systematic increases in the duration of forelimb tonus. Secondly, although the stimulations at 75 mA were considerably more intense than those previously used by Ramer and Pinel [illegible] same general pattern of kindling [illegible]

Figure 2. The incidence of individual alcohol withdrawal symptoms in the animals of Experiment 1. Each animal's score for a particular symptom was converted to a percentage of the mean of the control animal's scores for that symptom. The scores for each group reflect the mean of these converted scores.


PERCENT CONTROL MEAN
4000
2000
400
300
200
100
Control
Pseudo-ECS
15-mA ECS
75-mA ECS
Hyper-Reactivity
Ear-Eye Clonus
Jaw Clonus
Facial Clonus
Head Nods
Body Jerks
WITHDRAWAL SYMPTOMS

(1975), the same general pattern of kindling occurred. As in the Ramer and Pinel study, the higher intensity ECSs were found to produce more severe responses with each administration. However, in the present experiment, only the differences in forelimb extension latency were significant. Thirdly, because the animals of both groups were given test ECSs of standard intensity before and after kindling, direct comparisons could be made between these groups. These test ECSs made it clear that although the higher intensity stimulations appeared to produce more severe MSs, the degree of kindling produced was the same as that produced by the lower intensity stimulations.

The development of tolerance with exposure to alcohol and the subsequent convulsive withdrawal reactions were similar to those generally reported by other investigators (Majchrowicz, 1975). In this respect, the results of Experiment 1 were almost identical to those previously reported by Mucha, et al. (1975). The Mucha et al. (1975) study used exactly the same methods as those used in the present experiment and, in general, the same degree of tolerance, and the same type of withdrawal reaction wereproduced.

The major finding of this experiment was that periodic ECSs can potentiate a subsequent alcohol withdrawal syndrome. Such potentiation is consistent with other reports that periodic administration of one convulsive agent can increase the susceptibility of the animal to the the convulsive effects of another agent (Pinel, Skelton, & Mucha, 1975; Pinel & Van Oot, 1975). Although the rationale of Experiment 1 was based on the fact that kindling increases the seizure susceptibility

of the animal thereby increasing the susceptibility of the animal to the convulsive effects of alcohol withdrawal, the results of the present experiment offer another possible interpretation. The fact that higher doses of alcohol were required to initially induce and then maintain intoxication in the experimental animals suggests that the more severe withdrawal syndrome elicited in these animals might have been due to the greater amounts of alcohol they received. This implies that the role of repeated ECSs was not to increase the susceptibility to MSs per se, but rather to change the animal's tolerance of alcohol. By the methods of alcohol administration used in this experiment, these more tolerant animals would have been given greater amounts of alcohol than the unstimulated controls. This hypothesis of the relation between prior ECSs and alcohol tolerance was considered in the remaining four experiments of this thesis.

Both the ECS-kindling effect and the ECS-induced intensification of the alcohol withdrawal syndrome have several important clinical implications. Firstly, the fact that spontaneous seizures have been reported after a series of local brain stimulations (Rovner & Pinel, 1976; Wada & Sato, 1973) as well as after a series of clinical ECS treatments (Blumenthal, 1955; Folksman, 1947; Pacella & Barrera, 1945; Pollack, Rosenthal, & Macey, 1963) stresses the need for clinical investigation of the kindling effect. Secondly, the transfer of the increased seizure susceptibility produced by periodic ECSs could have hazardous consequences for certain patients. Until the appropriate tests have been conducted with human patients who have received ECSs,

the reaction of those patients to drugs, or to drug withdrawal should be closely monitored. There is no reason to suspect that the increased seizure susceptibility induced by repeated ECSs is specific to alcohol withdrawal. This is a particularly important point since patients receiving ECS treatments are also given a variety of drugs which can have convulsive effects either upon administration, or upon withdrawal (Goodman & Gilman, 1969; Kalinowsky & Hippius, 1969). Thus, extreme care should be taken not just with alcohol, but with these other drugs as well.

Experiment 2: Duration of the Inter-ECS Interval and the Intensification of the Alcohol Withdrawal Syndrome

Introduction

One purpose of Experiment 2 was to confirm the relation between inter-ECS interval and the facilitation or inhibition of ECS-kindling previously reported by Ramer and Pinel (1975). They found that when ECSs were presented at 3-day intervals, a kindling effect was produced, but when ECSs were presented at 1-hr intervals, there was a progressive inhibition of MSs. Thus, in the present experiment, ECSs were presented at 1-hr or 3-day intervals and the progressive effects on MS severity were assessed.

A second purpose of Experiment 2 was to determine the effects of inter-ECS intervals on the subsequent intensity of the alcohol withdrawal syndrome. In addition to providing important parametric information, this experiment compared the effects of ECSs on the severity of the alcohol withdrawal syndrome under two conditions, one in which ECSs produce MSs of increasing severity and the other in which they produce MSs of decreasing severity. The results of Ramer & Pinel (1975) provided the basis for this manipulation by changing the interval between stimulations. By using these two schedules of ECS presentation, the necessity of kindling *per se* in the intensification of the alcohol withdrawal syndrome might be determined.

Methods

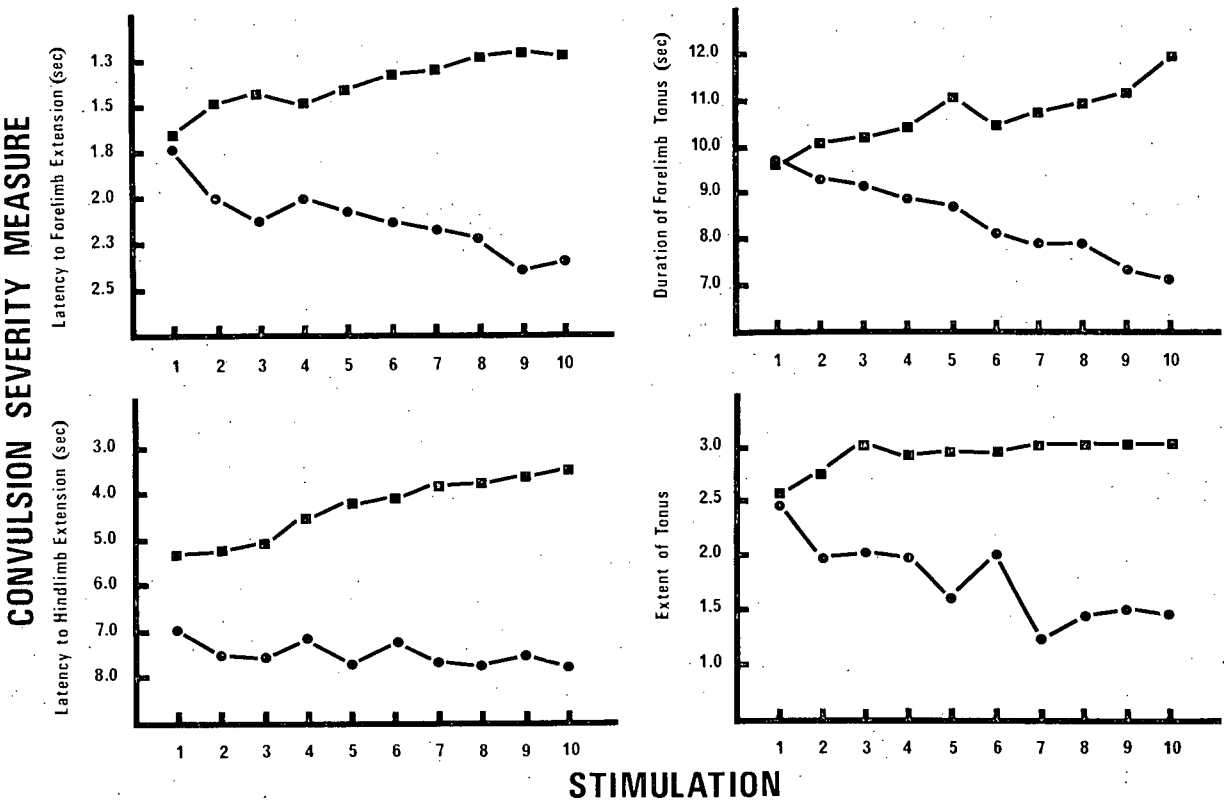
All 63 subjects were implanted with ECS electrodes before random assignment to one of the six groups. The rats in three of the groups were intubated with alcohol in the standard manner after ten, 15-mA ECSs presented at 3-day (n = 11) or 1-hr intervals (n = 10), or not at all (control; n = 10). Rats in the remaining three groups were stimulated at the same intervals (3 days, n = 10; 1 hr, n = 9; not at all, n = 10), but were subsequently intubated with 0.9% solutions of saline. Those animals not receiving ECSs were handled to the same degree as those receiving ECSs. After the last intubation, the incidence of withdrawal symptoms was assessed as before.

Results

When ECSs were presented at 3-day intervals there was a progressive intensification of the ECS-induced MSs, whereas in the 1-hr condition there was a progressive decline in the severity of the MSs. Furthermore, only the ECSs which were presented at 3-day intervals potentiated the alcohol withdrawal syndrome.

ECS-induced MSs. There was a progressive increase in the severity of the MSs elicited by ECSs presented once every 3 days (Figure 3); however, a progressive decrease in MS severity was observed in the 1-hr condition. The data for the two groups receiving ECSs once every 3 days and for the two groups receiving ECSs at 1-hr intervals

Figure 3. The progressive change in MS severity in rats receiving 15-mA ECSs at 3-day (squares) or 1-hr (circles) intervals.


CONVULSION SEVERITY MEASURE
Latency to Forelimb Extension (sec)
1.3
1.5
1.8
2.0
2.3
2.5
Duration of Forelimb Tonus (sec)
12.0
11.0
10.0
9.0
8.0
7.0
Latency to Hindlimb Extension (sec)
3.0
4.0
5.0
6.0
7.0
8.0
Extent of Tonus
3.0
2.5
2.0
1.5
1.0
1 2 3 4 5 6 7 8 9 10
STIMULATION

were combined for the analysis of changes in MS severity. A comparison of the severity of the first and last MSs of the 3-day group revealed a significant increase in all four measures of MS severity (all p's < 0.01). The progressive decrease in the severity of the MS elicited at 1-hr intervals was indicated by significant decreases in three of the measures of seizure severity; latency to forelimb tonic extension (F (1,18) = 5.63, $p < 0.05$), duration of forelimb tonus, (F (1,18) = 8.51, $p < 0.05$), and extent of tonic extension (Sign Test, N = 6, x = 0, $p < 0.05$).

The same pattern of significant changes was illustrated by between-group comparisons. The first MSs of the animals of the 1-hr and 3-day groups differed significantly on only the latency to hindlimb tonic extension (F (1,23) = 6.83, $p < 0.05$). However, the last MSs of the animals in the 3-day group were significantly more severe than those of the animals in the 1-hr group on all four measures of MS severity (all p's < 0.02).

Alcohol tolerance. Unlike the results of Experiment 1, there were no significant differences between any two of the three groups in terms of initial tolerance (F (2,32) = 0.27, $p > 0.05$), final tolerance (F (2,32) = 2.33, $p > 0.05$), or the change in tolerance (F (2.32) = 1.35, $p > 0.05$). The mean results for each of the groups are presented in Appendix I.

Withdrawal syndrome. The fact that the alcohol withdrawal syndromes of the animals not exposed to alcohol were significantly less

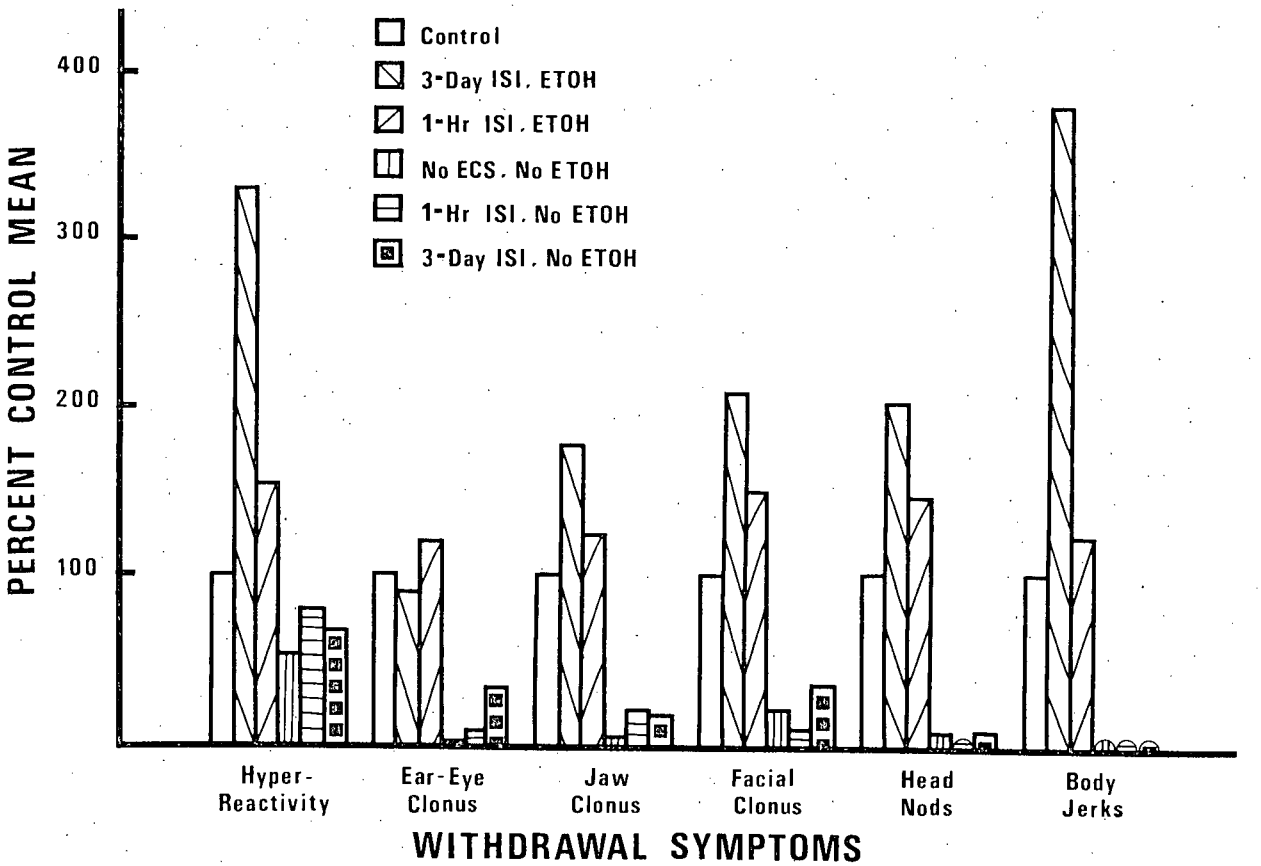
severe than those of the animals receiving alcohol (Figure 4) clearly illustrates the convulsive effects of alcohol withdrawal (all p's<0.01). Furthermore, ECSs alone had no effect on the incidence of these symptoms since the groups receiving ECSs but not alcohol did not display significantly different incidences of these symptoms than animals receiving neither alcohol nor ECSs (all p's>0.10).

The major finding of the present experiment was that the alcohol withdrawal syndrome was significantly potentiated by the ECSs presented once every 3 days, but not by those presented at 1-hr intervals (Figure 4). An overall analysis of variance performed on the withdrawal scores revealed a significant difference among the six groups (F (5,57) = 21.12, $p<0.01$). Subsequent individual comparisons revealed that the withdrawal syndrome of the 3-day animals was significantly more severe than that of either the 1-hr animals (F (1,19) = 12.49, $p<0.10$) or the control animals (F (1,22) = 18.30, $p<0.01$). Although it appears from Figure 4 as though some potentiation of the alcohol withdrawal syndrome may have been produced by the ECSs administered at 1-hr intervals, the difference between the animals of the 1-hr group and the control animals was not significant (F (1,21) = 1.33, $p>0.10$).

Discussion

The results of Experiment 2 confirmed Ramer and Pinel's (1975) report of the relation between the inter-ECS interval and the progressive changes in MS severity; stimulations presented once every 3 days

Figure 4. The incidence of individual alcohol withdrawal symptoms in the six groups of animals of Experiment 2. Each animal's score for a particular symptom was converted to a percentage of the mean of the control animals scores for that symptom. The scores for each group reflect the mean of these converted scores.


Control
3-Day ISI. ETOH
1-Hr ISI. ETOH
No ECS. No ETOH
1-Hr ISI. No ETOH
3-Day ISI. No ETOH
400
300
200
100
PERCENT CONTROL MEAN
Hyper-Reactivity
Ear-Eye Clonus
Jaw Clonus
Facial Clonus
Head Nods
Body Jerks
WITHDRAWAL SYMPTOMS

facilitated kindling whereas stimulations at 1-hr intervals inhibited MSs. Experiment 2, however, extended these findings by also showing changes in a fourth measure, the duration of forelimb tonic extension, which were consistent with the changes in the other three measures.

A similar relation has been reported in experiments in which animals have been kindled with amygdaloid stimulations; inhibition is produced with short intervals, whereas kindling occurs with longer intervals. The main difference between ECSs and local brain stimulations in this regard, however, appears to be that the inhibitory effects of seizures elicited by amygdaloid stimulation are much less enduring than those produced by seizures elicited by ECSs. For example, Goddard et al. (1969) found that kindling could be produced with amygdaloid stimulation at intervals of 20 min or more. If stimulations were presented at shorter intervals they would eventually fail to elicit MSs even in kindled animals. Ramer and Pinel (1975), however,found that ECS-kindling was produced with 3-day intervals and that if 1-hr intervals were used the severity of the MSs progressively decreased.

Unlike Experiment 1, no significant differences between any of the groups in initial tolerance, final tolerance, or the change in tolerance were found. The results of Experiment 1 suggested that the intensification of the alcohol withdrawal syndrome was due to changes in the animal's tolerance of alcohol which resulted in the experimental animals receiving greater amounts of alcohol than the unstimulated animals. However, in the present experiment, and in all the remaining experiments, there were no differences in the tolerance of the animals

of alcohol. Therefore, it appears more likely that the potentiation of the alcohol withdrawal syndrome was due to an increase in the animal's susceptibility to the convulsive effects of alcohol withdrawal produced by the periodic ECSs.

The fact that the alcohol withdrawal syndrome was intensified only when ECSs were presented at 3-day, but not 1-hr intervals suggests that ECSs per se are not responsible for the intensification effect. The findings of Experiment 2 suggest that the same effects which induce the progressive increase in MS severity produced by periodic ECSs also form the basis for the intensification of the alcohol withdrawal syndrome. This interpretation, would suggest that the inhibitory effects of ECSs presented at 1-hr intervals should reduce the severity of the alcohol withdrawal syndrome. However, whereas the increases in seizure susceptibility associated with kindling with other agents have been shown to be relatively enduring (Goddard et al., 1969; Mason and Cooper, 1972), the inhibitory effect of seizures have been shown to be relatively short-lived (Mucha, Pinel, & Phillips, 1976). It is perhaps for this reason that no diminution of the alcohol withdrawal syndrome was observed in the animals presented with ECSs at 1-hr intervals.

Experiment 3: Number of ECSs and the Intensification of the Alcohol Withdrawal Syndrome

Introduction

The purpose of Experiment 3 was to determine the relation between the number of ECSs and both the degree of ECS-kindling and the subsequent intensification of the alcohol withdrawal syndrome.

Methods

Following post-surgical recovery, the 51 subjects were randomly divided into groups which received 20 (n = 10), 10 (n = 9), 6 (n = 9), 3 (n = 9), or no ECSs (control; n = 13). The 15-mA ECSs were presented once every 3 days as before, but only the MSs of the animals receiving 20 stimulations were videotaped and analyzed. The onset of the stimulations was staggered so that alcohol exposure terminated on the same day for all animals. Alcohol administrations and withdrawal observations were as before.

Results

Both the progressive intensification of the ECS-induced MSs and the intensification of the alcohol withdrawal syndrome were found to be increasing, negatively-accelerated functions of the number of ECSs.

ECS-induced MSs. As in the previous experiments, the MSs grew progressively more severe in response to each successive ECS (Figure 5). Although there was some variability in the course of the intensification of the four measures of MS severity, in general, the sixth MSs were significantly more severe than the first in terms of all four measures of MS severity (all p's < 0.05). An analysis of all four measures of MS severity indicated that the tenth MS was more severe than the first (all p's < 0.05). However, the 20th MSs were not significantly more severe than the tenth on any of the four measures. (all p's > 0.05).

Alcohol Tolerance. As in Experiment 2, there were no significant overall differences between the groups in initial tolerance (F (4,43) = 0.91, $p > 0.05$), final tolerance (F (4,43) = 0.92, $p > 0.05$), or the change in tolerance (F (4,43) = 0.51, $p > 0.05$). The mean values for these three measures for each group are presented in Appendix I.

Withdrawal syndrome. The severity of the alcohol withdrawal syndrome was a function of the number of ECSs (Figure 6). An overall analysis of variance indicated a highly significant difference among the animals of the five groups (F (4,43) = 17.55, $p < 0.01$). Subsequent individual comparisons revealed that the incidence of withdrawal symptoms was significantly greater in the animals of the 20-ECS group than in the animals of either the 6-ECS, (F (1,17) = 74.03, $p < 0.01$), 3-ECS (F (1,17) = 26.24, $p < 0.01$) or control groups (F (1,21) = 58.13, $p < 0.01$). Moreover, the incidence of withdrawal symptoms was signifi-

Figure 5. The progressive increase in MS severity produced by 20, 15-mA ECSs presented at 3-day intervals.

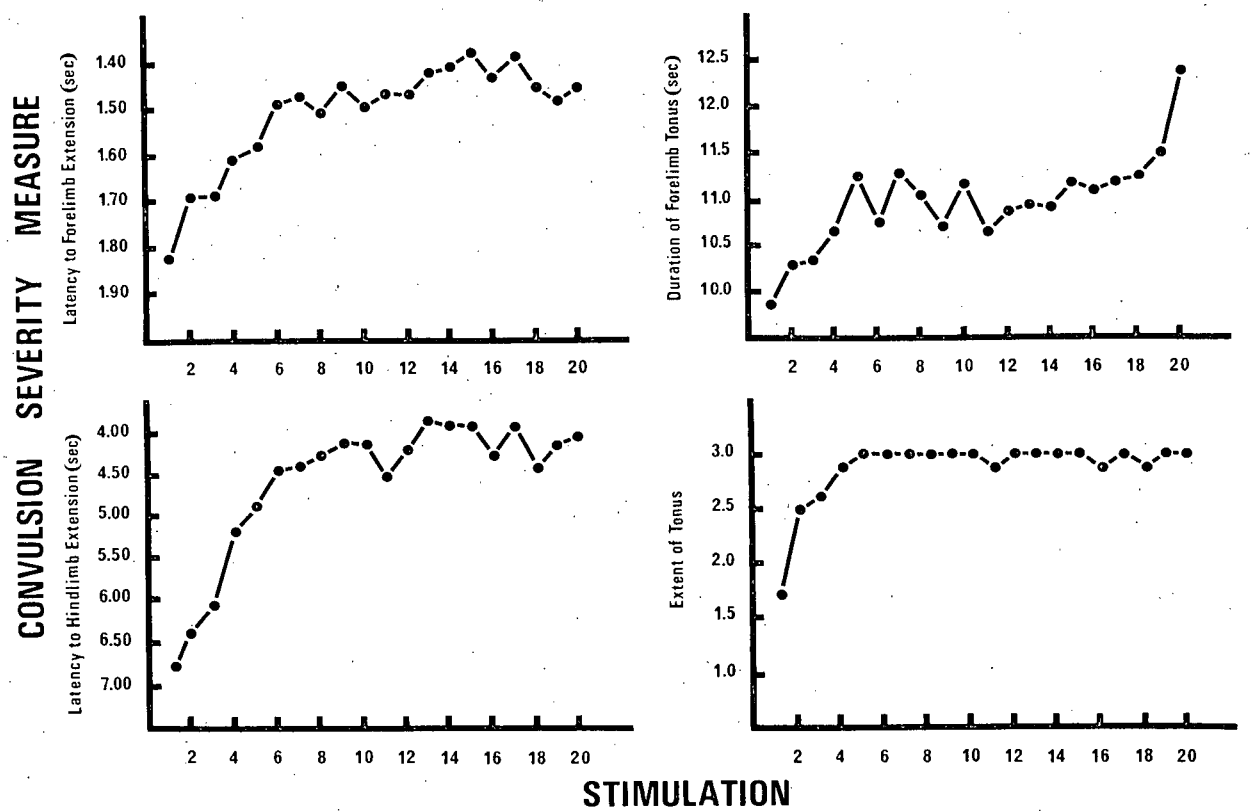
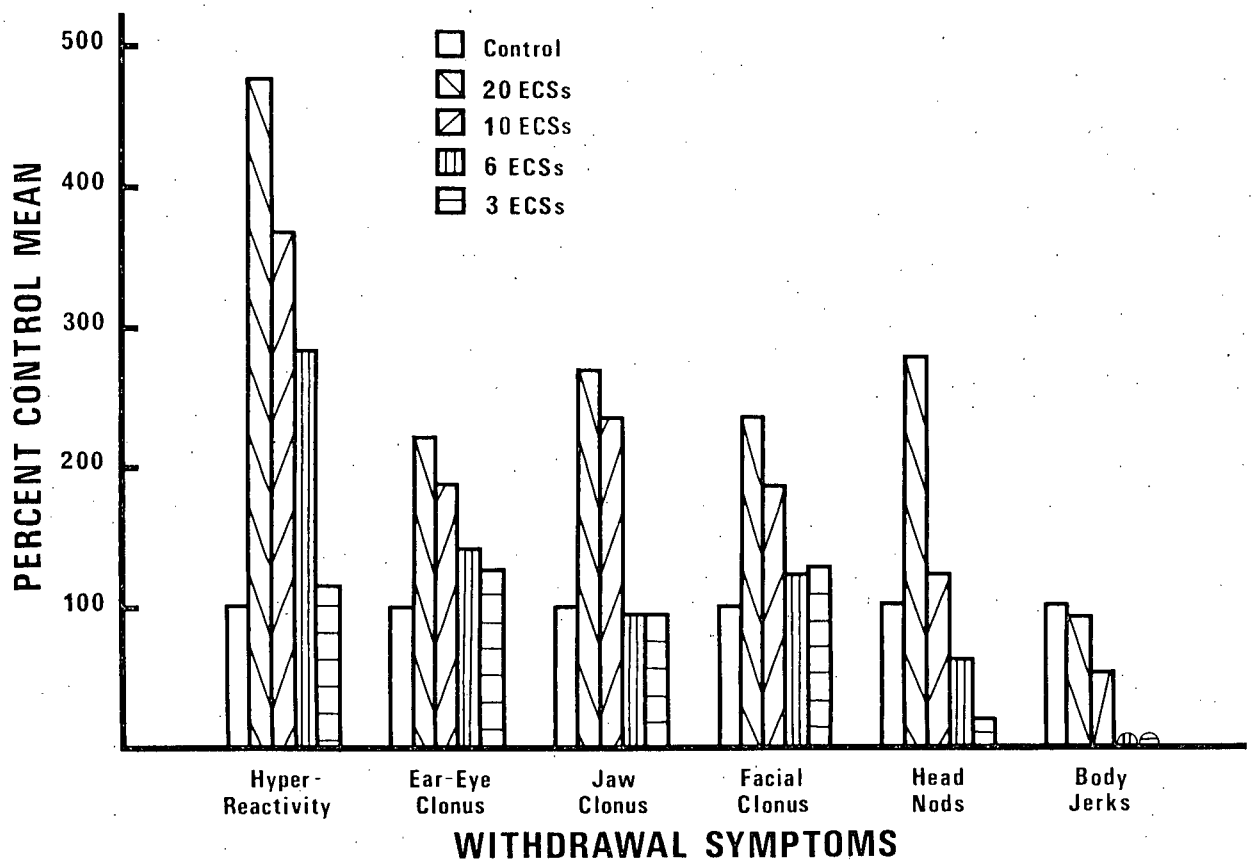

CONVULSION SEVERITY MEASURE
Latency to Forelimb Extension (sec)
1.40
1.50
1.60
1.70
1.80
1.90
Duration of Forelimb Tonus (sec)
12.5
12.0
11.5
11.0
10.5
10.0
Latency to Hindlimb Extension (sec)
4.00
4.50
5.00
5.50
6.00
6.50
7.00
Extent of Tonus
3.0
2.5
2.0
1.5
1.0
2 4 6 8 10 12 14 16 18 20
STIMULATION

Figure 6. The incidence of individual alcohol withdrawal symptoms in the five groups of animals in Experiment 3. Each animal's score for a particular symptom was converted to a percentage of the mean of the control animal's scores for that symptom. The scores of each group reflect the mean of those converted scores.


500
400
300
200
100
PERCENT CONTROL MEAN
Control
20 ECSs
10 ECSs
6 ECSs
3 ECSs
Hyper-Reactivity
Ear-Eye Clonus
Jaw Clonus
Facial Clonus
Head Nods
Body Jerks
WITHDRAWAL SYMPTOMS

cantly greater in the animals of the 10-ECS group than that of the animals of the 6-ECS (F (1,16) = 15.03, $p < 0.01$) and control groups (F (1,20) = 11.86, $p < 0.05$). None of the other differences between groups were significant (all p's > 0.10).

Discussion

As in the previous experiments 10 ECSs significantly potentiated the alcohol withdrawal syndrome. Experiment 3 also contributed to the findings of the two previous experiments by documenting the relation between the number of ECSs and the degree of severity of the alcohol withdrawal syndrome. In Experiment 3, six or fewer ECSs were not sufficient to significantly intensify the alcohol withdrawal syndrome, whereas 10 or more were. The present results suggest that in subsequent attempts to determine whether or not the susceptibility to alcohol withdrawal is increased by clinical ECS treatments, attention should be directed toward those patients who have had large numbers of ECSs. The results of Experiment 3 have also shown that the degree of MS severity is directly related to the number of ECSs in a series. Although each measure of MS severity developed at its own rate, in general MS severity was more severe after large numbers of ECSs. However, as can be seen from Figure 5, the degree of intensification appears to be asymptotic by approximately the tenth ECS; there was a slight, but non-significant increase in the severity of each of the four measures of MS severity from the tenth to the 20th MSs. It would

appear from these data that the course of ECS-kindling is complete after 10 stimulations. However, it is difficult to rule out the possibility that important developments in epileptogenesis might occur with additional ECSs. This possibility is illustrated by the development of spontaneous seizures with local electrical brain stimulations. In the majority of kindling studies very few stimulations have been commonly used, largely because local brain stimulations produce MSs which intensify and develop very quickly. However, recent studies have revealed that striking changes can be observed after 200 to 300 stimulations in the form of interictal spiking and the development of spontaneous seizures (Pinel & Rovner, 1976; Wada & Sato, 1974). Some clinical evidence even exists that with a large number of ECSs spontaneous seizures might occur (Pacella & Barrera, 1945; Pollack, Rosenthal, & Macey, 1963). Thus, it may be premature to rule out the possibility that ECS-kindling does not continue to progress with more than 10 stimulations.

Experiment 4: Permanence of the ECS-Induced Intensification of the Alcohol Withdrawal Syndrome

Introduction

In each of the previous three experiments periodic ECSs intensified the alcohol withdrawal syndrome. The purpose of Experiment 4 was to assess the permanence of this effect.

Methods

Of the 49 subjects, 36 received ten 15-mA ECSs at 3-day intervals. These animals were randomly divided into four groups with delays of 10 weeks (n = 7), 6 weeks (n = 10), 3 weeks (n = 10), or 2 weeks (n = 9) between the last ECS and the termination of alcohol exposure. The remaining 13 animals (controls) were also intubated with alcohol but remained unstimulated. The onset of the ECS administrations was again staggered so that each animal was withdrawn from alcohol on the same day. Since the ECS-kindling effect initially reported by Ramer and Pinel (1975) was replicated in each of the first three experiments of this thesis, the ECS-kindling effect was not assessed as thoroughly in this experiment. However, in this experiment, the extent of hindlimb extension was monitored to confirm the development of progressively more severe MSs.

Results

As in Experiments 1, 2, and 3, ECS-kindling occurred in response to the periodic ECSs. Although the same degree of tolerance was displayed in all groups, the animals which received ECSs suffered a more severe withdrawal syndrome. However, the degree of intensification of the withdrawal syndrome was inversely related to the delay between the last ECS and the termination of alcohol exposure. A significant degree of intensification lasted up to 3 weeks after the last ECS.

ECS-induced MSs. To the extent that it was measured, the progressive increase in the severity of MSs was largely the same as that observed in the previous experiments. In response to the first ECS approximately 40% of the animals did not display maximal hindlimb extension, whereas, in response to the last stimulation, all animals displayed full extension.

Alcohol tolerance. As in Experiments 2 and 3, there were no significant differences between the groups in their tolerance to alcohol. There were no significant differences in their initial tolerance (F (4,44) = 1.52, $p > 0.05$), their final tolerance (F (4,44) = 1.26, $p > 0.05$), or their change in tolerance (F (4,44) = 1.58, $p > 0.05$). The means of each group for these measures are presented in Appendix I.

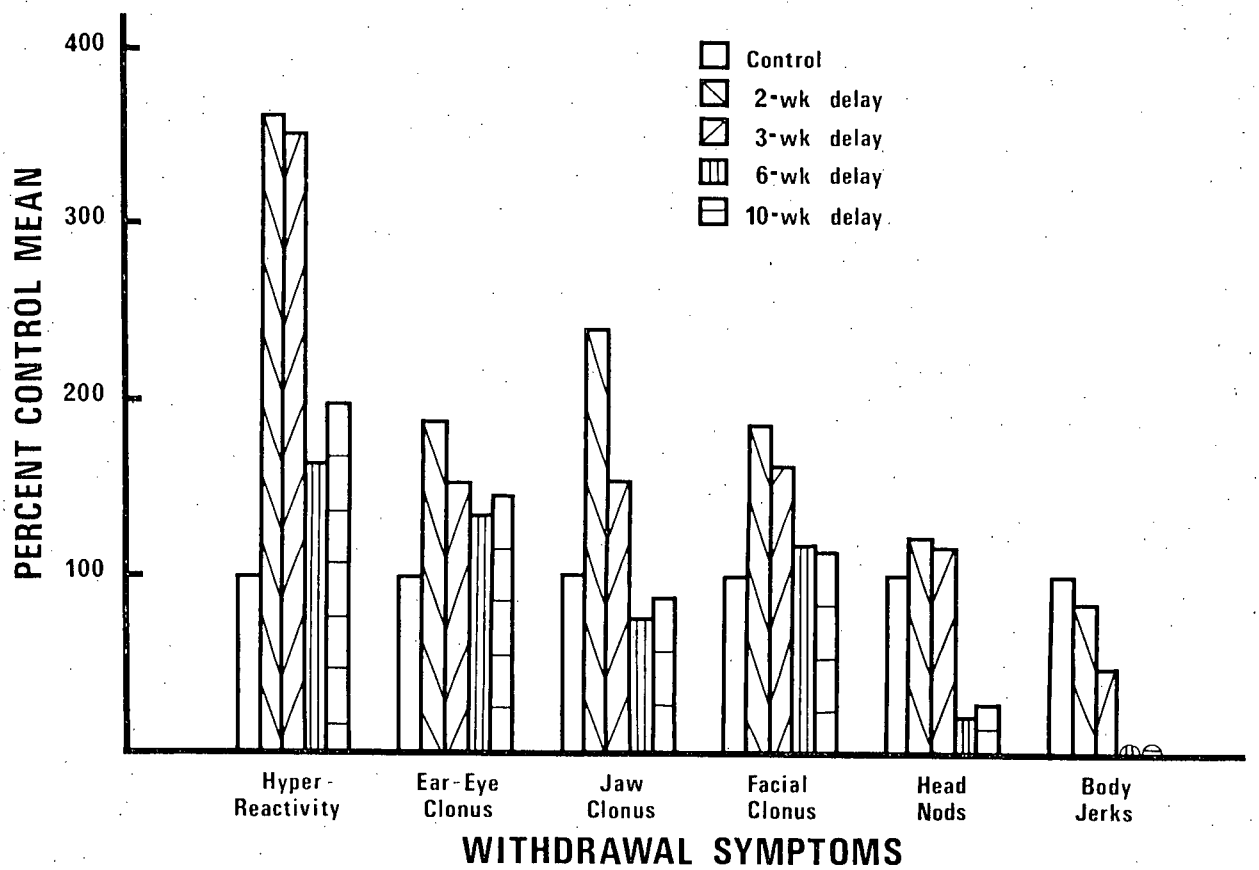
Withdrawal syndrome. The degree of intensification of the alcohol

withdrawal syndrome was inversely related to the length of the delay between the last ECS and the termination of alcohol exposure (Figure7). As in Experiments 1, 2, and 3, ten periodic ECSs significantly intensified the reaction of the animals to alcohol withdrawal 2 weeks later. However, when the delay between the last ECS and the termination of alcohol exposure was longer the severity of the subsequent alcohol withdrawal syndrome was less. Relative to the controls, the incidence of combined withdrawal symptoms was significantly higher in the animals of the 2-week (F (1,20) = 15.31, $p < 0.01$) and 3-week delay groups (F (1,21) = 10.55, $p < 0.05$). However, animals that were tested at longer intervals did not differ significantly from the controls (all p's > 0.10). The inverse relation between the delay and the severity of the withdrawal syndrome was also illustrated by differences between the experimental groups. The incidence of combined withdrawal scores was significantly greater in the animals of the 2-week delay group than that in the animals of the 6-week (F (1,17) = 17.29, $p < 0.01$) and 10-week delay groups (F (1,21) = 11.95, $p < 0.05$). However, the incidence of combined withdrawal scores in the animals of the 3-week delay group was significantly greater than that in the animals of the 6-week delay group only (F (1,18) = 10.73, $p < 0.05$).

Discussion

The intensification of the alcohol withdrawal syndrome by prior ECS appears to be a decreasing function of the delay between the last ECS and alcohol withdrawal. Under the conditions of the present experiment, the intensification of the alcohol withdrawal syndrome was not observed after delays of 6 weeks or more.

Figure 7. The incidence of individual alcohol withdrawal symptoms in the animals of Experiment 4. Each animal's score for a particular symptom was converted to a percentage of the mean of the control animal's score for that symptom. The scores for each group reflect the mean of these converted scores.


400
300
200
100
PERCENT CONTROL MEAN
Control
2-wk delay
3-wk delay
6-wk delay
10-wk delay
Hyper-Reactivity
Ear-Eye Clonus
Jaw Clonus
Facial Clonus
Head Nods
Body Jerks
WITHDRAWAL SYMPTOMS

ECSs have been shown to have biochemical and neurophysiological effects which follow a time course similar to that reported in the present experiment for the intensification of the alcohol withdrawal syndrome. For example, increases in monoamine oxidase activity have been reported to last 6 weeks after a series of 42 ECSs in rats (Pryor & Otis, 1970). Moreover, after a comparable number of ECSs, adverse effects on normal growth patterns in young rats have been reported to last more than 2 weeks (Pryor, 1974). McGaugh (1974) also found that, depending on the parameters of the ECS presentation, retrograde amnesia in animals can last from 12 hr to 1 month. In humans, indication of EEG slowing after approximately 15 ECSs has been reported to last up to 2 or 3 months (Small, 1974). The relation of the time course of these effects and the intensification of the alcohol withdrawal syndrome described in the present experiment leads one to speculate that one of the changes underlying these effects might also be the basis of the intensification effect. Indeed, attempts have already been made to establish the effects of the interaction of ECS and various neurotransmitters on the seizure susceptibility of organisms (Ottoson, 1960; Karczmar, 1974). However, to establish the relation between these effects and the ECS-induced intensification of the alcohol withdrawal syndrome, studies using conditions comparable to those used in the present experiment, will have to be conducted.

Experiment 5: Drug Pretreatments and ECS-Induced Intensification of the Alcohol Withdrawal Syndrome

Introduction

The purpose of this fifth and final experiment was to assess the effects of a series of drugs routinely administered in conjunction with clinical ECS treatments on the ECS-induced intensification of the alcohol withdrawal syndrome.

Because of certain hazards associated with ECS treatments, patients typically receive three different drugs prior to each treatment; atropine sulfate, a barbiturate, and succinyl-choline, (Fink, 1974; Frankel, 1973). Patients receiving several ECS administrations frequently complain of severe anxiety before and after each ECS. Barbiturates have been found to reduce this anxiety. Atropine sulfate, on the other hand, has been found to be effective in reducing the problems of apnea and bradycardia which sometimes occur after an ECS-induced MS. One of the most common complaints and hazards associated with ECS administrations is that of bone fractures or dislocations produced by the MS. Succinyl-choline has been found very effective in reducing these hazards by blocking muscle contractions. In some cases oxygen is also administered to reduce the problems associated with anoxia sometimes occurring as a result of the elicited convulsions. Thus, in Experiment 5 each of these four agents was given to the experimental animals prior to each ECS to assess the effects of these

drugs on the ECS-induced intensification of the alcohol withdrawal syndrome.

Methods

The 39 subjects were randomly divided into four groups, one receiving the series of drugs prior to each ECS (n = 10), another receiving the drugs, but no ECSs (n = 8), a third receiving only the ECSs (n = 12), and a fourth receiving neither the drugs nor the ECSs (control; n = 9). Those animals not receiving ECSs received control handling. Thus, the design was a 2x2 factorial with the two variables being ECS administrations and drug pretreatments with all the animals receiving alcohol. Prior to each scheduled ECS, the "drug" subjects received i.p. injections of atropine sulfate (0.036 mg/kg), sodium pentobarbital (50 mg/kg), and succinyl-choline (10 mg/kg). The concentrations of each aqueous drug solution were adjusted so that each animal received about 0.2 cc of solution per injection. The atropine, barbiturate and succinyl-choline were administered 30, 15, and 3 minutes prior to each ECS respectively. Each animal was then oxygenated for 30 sec immediately prior to each ECS in a low-pressure oxygen chamber (45x45x90 cm). After each ECS, any animal displaying breathing difficulties producing marked cyanosis of the hindlimbs was returned to the oxygen chamber for 1 min. However, if an animal ceased breathing altogether, it was resuscitated on a Harvard Small-Animal Resuscitator until normal breathing returned. Only eight of the 18 animals which

received drugs required additional oxygenation at some time during the experiment, but only 3 of the 8 required resuscitation. There was no relation between these treatments and later responses to alcohol or alcohol withdrawal.

As usual, 1 day after the last ECS, alcohol was administered for 14 days. After this alcohol intubation period the severity of the alcohol withdrawal syndrome was assessed as before.

Results

Although the drug treatments eliminated any overt convulsive responses to the ECSs, the usual ECS-induced intensification of the alcohol withdrawal syndrome was not significantly reduced by these pretreatments.

ECS-induced MSs. The drugs administered prior to each ECS rendered the animals completely ataxic, and sedated. With the application of current, a mild jerk or tremor ran through the body which did not outlast the duration of the stimulation. Within 20 min of each ECS, all animals were awake and behaving normally with no apparent after-effects.

Alcohol tolerance. As in the case of the previous three experiments no significant differences between any of the groups were found on initial tolerance (F (3,35) = 1.56, $p > 0.05$), final tolerance (F (3,35) = 1.12, $p > 0.05$) or the change in tolerance (F (3,35) = 2.39, $p < 0.05$), but

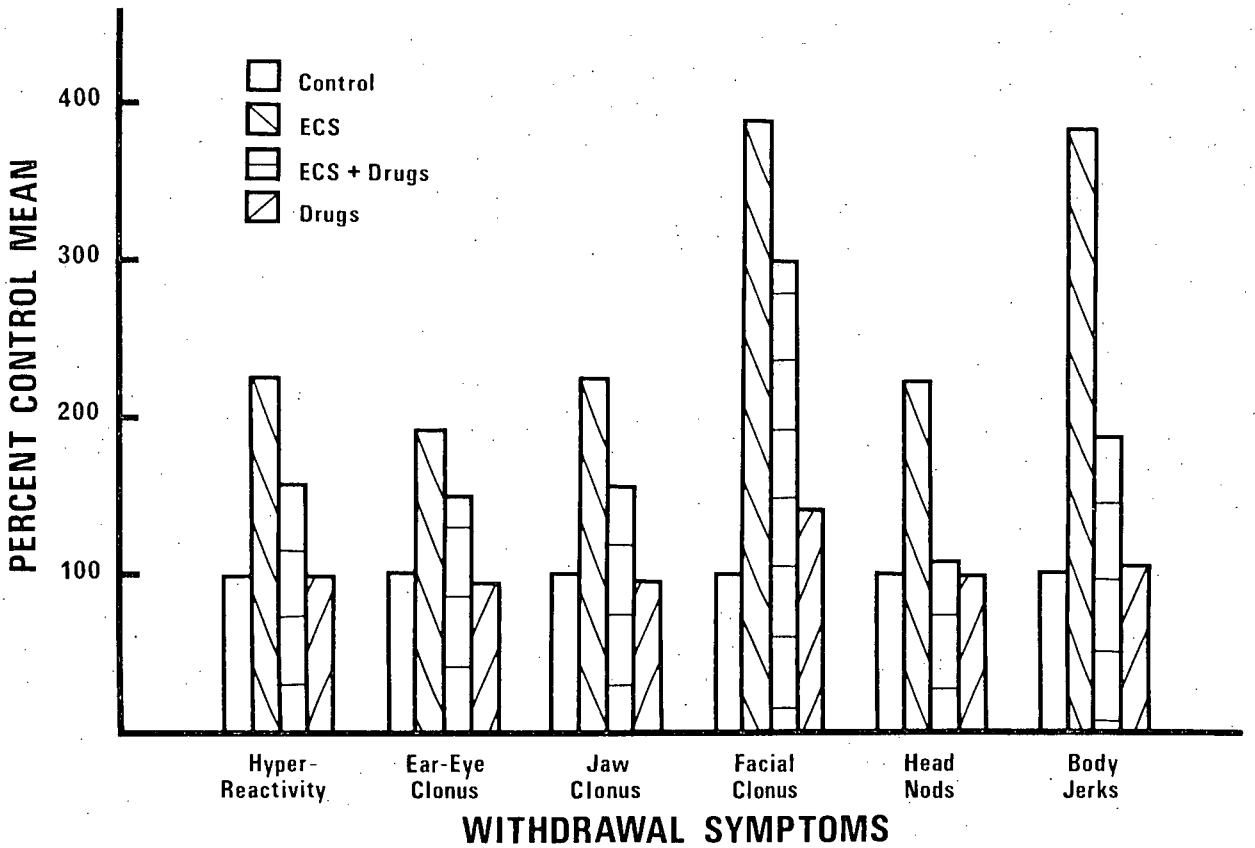
$p > 0.05$). All means are again presented in Appendix I. Thus, as in the previous three experiments, the ECSs had no effect on the tolerance of the animal of alcohol.

Withdrawal syndrome. As in the previous experiments, an intensification of the alcohol withdrawal syndrome was observed in subjects receiving 10 ECSs (Figure 9). Moreover, this intensification occurred in animals whether or not their MSs had been pharmacologically blocked. An overall analysis of variance revealed significant differences among the four groups in the combined withdrawal scores (F (3,35) = 11.89, $p < 0.01$). Subsequent individual comparisons revealed that the subjects receiving ECSs either alone or after the drug pretreatments displayed a higher incidence of combined withdrawal symptoms than did the rats in either the control (F (1,19) = 18.54, $p < 0.01$, and F (1,17) = 14.57, $p < 0.01$, respectively) or drug-no EEG conditions (F (1,18) = 14.67, $p < 0.01$, and F (1,16) = 10.89, $p < 0.025$, respectively). The drug treatments themselves had no significant effect on the withdrawal scores regardless of whether they were administered in conjunction with ECSs (F (1,20) = 4.22, $p > 0.10$) or not (F (1,15) = 1.48, $p > 0.10$).

Discussion

The results of Experiment 5 thus confirm those of the other four experiments of this thesis; an intensification of the alcohol withdrawal syndrome was produced by 10 periodic ECSs. More importantly,

Figure 8. The incidence of individual alcohol withdrawal symptoms for the four groups of animals in Experiment 5. Each animal's score for a particular symptom was converted to a percentage of the mean of the control animals for that symptom. The scores for each group reflect the mean of these converted scores.


Control
ECS
ECS + Drugs
Drugs
PERCENT CONTROL MEAN
400
300
200
100
Hyper-Reactivity
Ear-Eye Clonus
Jaw Clonus
Facial Clonus
Head Nods
Body Jerks
WITHDRAWAL SYMPTOMS

however, was the finding that the elicitation of MSs typically produced by ECSs is not necessary for increasing the severity of the withdrawal syndrome. This is consistent with the report that periodic amygdaloid stimulations can increase the severity of a subsequent alcohol withdrawal syndrome, even when the stimulation intensities are too low to elicit MSs (Pinel, Van Oot & Mucha, 1975).

In the study by Pinel et al. (1975), amygdaloid stimulations which were not intense enough to elicit MSs, produced a potentiation of the alcohol withdrawal syndrome which was slightly, but not significantly less than that produced by stimulations which reliably elicited MSs. This same relation was also observed in the findings of Experiment 5. Thus, periodic ECSs can potentiate the alcohol withdrawal syndrome even when the MSs are pharmacologically blocked. The elicitation of MSs, however, does appear to contribute to the degree of intensification in some way.

Discussion

In the present experiments periodic ECSs were found to intensify an animal's reaction to subsequent ECSs and to potentiate the convulsive symptoms of alcohol withdrawal. The intensification of MSs by periodic ECSs (ECS-kindling) was shown to be a function of the inter-ECS interval and the number of ECSs, but was found not to be affected by current intensity. Furthermore, when ECSs were administered at 3-day intervals, the elicited MSs became progressively more severe; whereas with 1-hr intervals, the MSs became progressively less severe. When elicited once every 3 days, the MSs increased in intensity with each successive ECS; however, there were only minor increases after the tenth stimulation. Finally, the magnitude of the change in MS severity was the same at either 15 or 75 mA even though the higher intensity current consistently appeared to elicit more severe MSs. These same variables influenced the degree to which periodic ECSs potentiated the reaction to subsequent alcohol withdrawal; ECSs administered at 3-day intervals increased the incidence of withdrawal symptoms, whereas those presented at 1-hr intervals did not. The intensification of the alcohol withdrawal syndrome also became more marked with greater numbers of ECSs and was found to be equally as severe after ECSs of either 15 or 75 mA. Two additional variables were investigated with regard to the ECS-induced potentiation of the alcohol withdrawal syndrome. Under the conditions used in these experiments subjects were rendered more susceptible to the convulsive effects of alcohol withdrawal for a period of several

weeks after the last of a series of ten ECSs. Also, this intensification was found to occur even though the MSs typically elicited by ECSs had been pharmacologically blocked.

There are a number of similarities between the progressive changes in MS severity observed during the course of the present investigations and those produced by a series of local brain stimulations (kindling). Firstly, in both cases there is a progressive increase in the severity of the elicited MSs. In this regard, kindling with neocortical stimulations is more like kindling produced with repeated ECSs; MSs are typically elicited on the first stimulation and become progressively more severe with subsequent stimulations. It may be recalled that with subcortical stimulations, there are no behavioural responses to the first few stimulations. Secondly, for both ECS-kindling, and kindling with amygdaloid stimulations, kindling progresses more rapidly at long intervals, with short intervals inhibiting MSs. The critical intervals for periodic ECSs seems to be longer,however. Although animals kindle effectively at 1-hr intervals with amygdaloid stimulations (Racine, et al., 1973), with ECSs intervals of greater than a day are required. When ECSs are presented at 1-hr intervals, there is a progressive decrease in the severity of MSs. Thirdly, either periodic ECSs or periodic amygdaloid stimulations produce increases in seizure susceptibility which are not specific to the agent used. Thus,animals kindled with either agent may be rendered more susceptible to the effects of other convulsive agents as well. In addition to the effects produced by periodic ECSs on the alcohol withdrawal syndrome documented

here, antecedent, periodic ECSs have also been found to intensify the convulsive effects of fluorothyl (Prichard, Gallagher, & Glaser, 1969). Periodic amygdaloid stimulations, on the other hand, have been reported to intensify the convulsive response to alcohol withdrawal (Pinel, Van Oot, & Mucha, 1975) and metrazol (Pinel & Van Oot, 1975). A fourth apparent similarity between kindling with periodic ECSs and amygdaloid stimulations is that the effects of both are relatively enduring. Because of the differences in the measures used, however, it is difficult to make precise comparisons between these two agents. In the present investigations, after ten ECSs, the alcohol withdrawal syndrome was significantly intensified 3 weeks, but not 6 weeks after the last ECS. Goddard et al. (1969) used a savings measure to assess the permanence of amygdaloid kindling and found a savings of 90% in the number of stimulations required to elicit MSs after a 12-week stimulation-free period. A fifth and final similarity is that the development of spontaneously recurring seizures has been reported with both periodic ECSs (Essig, et al., 1961; Pacella & Barrera, 1945; Pollack, Rosenthal and Macey, 1963) and local brain stimulations (Rovner & Pinel, 1976; Wada & Sato, 1974; Wada et al., 1975). These five similarities suggest that similar mechanisms may underlie kindling with these two agents. Thus insights into the mechanisms underlying the kindling phenomenon itself might be obtained by a comparison of kindling with these two agents. Moreover, attempts to explain the kindling phenomenon must now account for more than the features of amygdaloid kindling exclusively.

Although the results of the present investigations cannot be applied directly to clinical situations, they do suggest that the usual hazards of the alcohol withdrawal syndrome might be potentiated in humans after a series of ECSs. The results also suggest that this effect might be more marked after many ECSs have been administered at relatively long intervals and that the effect might also last several weeks after the termination of the treatments. Finally, the findings suggest that this intensification might occur even when the MSs of the ECSs have been pharmacologically blocked. Although these experiments do not prove that such hazardous interactions are associated with clinical ECS treatments, they do stress the need for research conducted in a clinical setting.

Although the alcohol withdrawal syndrome was the only agent monitored in the present investigations, there is not reason to believe that the effects of the increase in seizure susceptibility induced by periodic ECSs are specific to alcohol withdrawal. Many of the drugs frequently administered to patients undergoing ECS treatments have mildly convulsive effects either upon administration or withdrawal (Goodman & Gilman, 1969). It is, therefore, possible that a series of ECSs could potentiate the convulsive effects of one of these agents. For example, the withdrawal reaction after abrupt discontinuation of any one of the commonly-used dibenzazepines (e.g. imipramine or amytriptyline) could be intensified or perhaps the convulsive effects of high doses of the MAO inhibitors (e.g. iproniazide or pargyline) might be potentiated. Thus, potentially convulsive agents which are relatively

safe when administered in small doses may, in fact, elicit overt convulsive symptoms when administered following periodic ECSs. The use of these potentially convulsive drugs should, therefore, be carefully controlled and monitored after a series of periodic ECS administrations. Even though all drug treatments are typically discontinued during the course of ECS treatments, because the effects of ECS-kindling are relatively enduring, the potentially hazardous effects of periodic ECSs may last into the next drug administration period.

An examination of the clinical ECS literature reveals several interesting parallels between the conditions described in this thesis for maximal intensification of the alcohol withdrawal syndrome by ECS and those which are assumed to produce optimal therapeutic effects. Firstly, in Experiment 1 it was found that the degree of the intensification of the alcohol withdrawal syndrome was the same after periodic ECSs of two different suprathreshold current intensities. It is generally accepted that the therapeutic effects of clinical ECSs lie in the epileptiform electrographic discharges and do not depend on the level of the current as long as these ECSs elicit seizures (cf. Fink, 1974). Kalinowsky and Hippius (1969) and Ottoson (1960), for example, have argued that ECSs at intensities which do not elicit electrographic seizures do not produce therapeutic effects whereas a number of different supra-maximal intensities ECSs do not differ in their therapeutic effects. Secondly, in Experiment 2 the intensification of the alcohol withdrawal syndrome was produced only when animals received ECSs presented at 3-day intervals whereas ECSs presented at shorter intervals

produced no observable effects on the withdrawal syndrome. Several authors have recommended that, for optimal therapeutic effects, ECSs should be presented from two to three times per week (Abrams & Fink, 1972; Frankel, 1973; Kalinowsky & Hippius, 1969). Abrams and Fink (1972), for example, concluded that the intervals necessary for the greatest behavioural effects are from 3 to 9 days with at least 24 to 48 hr between each ECS. When ECSs are presented at intervals of less than 1 hr, a very transient state of "regression" is produced in which a general inhibition of both the MSs and spontaneous behaviours in general, is produced (Kalinowsky & Hippius, 1969), and the therapeutic effects associated with distributed ECSs are apparently absent (Abrams & Fink, 1972). Thirdly, in Experiment 3, the degree of the intensification of the alcohol withdrawal syndrome was found to be an increasing, negatively-accelerated function of the number of antecedent ECSs. In clinical practice, it is generally accepted that the larger the number of ECSs, the greater the therapeutic effects even though the exact number of stimulations is assumed to depend on the particular patient and disorder (cf. Fink, 1974). Fourthly, the results of Experiment 4 demonstrated that the increased susceptibility to convulsive withdrawal symptoms persisted for several weeks. Most clinical reports claim that the therapeutic effects of repeated ECSs are also relatively enduring. Finally, in Experiment 5, pharmacological pretreatment did not significantly reduce the intensification of the alcohol withdrawal syndrome. Frankel (1973) and Kalinowsky and Hippius (1969) have argued that the same series of drug pretreatments does not alter the thera-

peutic effects of repeated ECSs. These five parallels suggest that the conditions which produce optimal therapeutic effects are also those which maximize the hazards associated with the ECS-kindling effect. Moreover, the parallels suggest that the therapeutic and kindling effects may have a common basis. However, until direct comparisons are made between these two effects and on the parameters that influence them, this possibility is little more than speculation.

There are two major issues concerning the mechanisms underlying the ECS-induced intensification of the alcohol withdrawal syndrome. The first is whether the ECSs directly increase the seizure susceptibility of the animal or whether the intensification of the alcohol withdrawal syndrome is mediated by a change in alcohol tolerance. In other words, is the alcohol withdrawal syndrome intensified because the animals are more susceptible to the convulsive effects of alcohol withdrawal or do they suffer more severe withdrawal because they receive greater amounts of alcohol? The results of Experiment 1 seemed to support the latter hypothesis. In Experiment 1 animals which received ECSs were more tolerant of alcohol and thus required greater amounts of alcohol to produce the required degree of intoxication. Thus,it was not surprizing when these animals displayed more severe withdrawal than animals receiving less alcohol. However, in the remaining four experiments this relation between alcohol tolerance and ECSs was not replicated, it would seem that this hypothesis concerning the basis of the intensification effect is very unlikely. Thus, at this point, the most reasonable hypothesis is that repeated ECSs

directly increase the susceptibility of the animals to the convulsive effects of alcohol withdrawal.

A second major issue concerning the mechanisms underlying the ECS-induced intensification of the alcohol withdrawal syndrome is whether this effect is a product of kindling per se or rather a product of only the repeated ECSs. There are two findings in the present experiments which suggest that kindling may be the basis of the intensification of the alcohol withdrawal syndrome. In Experiment 2, two groups of animals received the same number of stimulations at two different intervals and had the same number of MSs, but only the animals in the group which displayed kindling also displayed an intensification of the alcohol withdrawal syndrome. In Experiment 3, only those animals in the groups which received a number of ECSs sufficient to produce a significant degree of ECS-kindling displayed a significant degree of intensification of the alcohol withdrawal syndrome. If the hypothesis was true that the intensification of the alcohol withdrawal syndrome was a product of ECS-kindling, then it should also have been true that the animals in an ECS group which were kindled should have been more responsive to alcohol withdrawal than those animals, in the same groups, which did not kindle. However, within-group analyses failed to confirm this prediction. No significant correlations were found between any of the measures associated with ECS-induced MSs and either the tolerance or withdrawal measures, or between any of the tolerance and withdrawal-related measures (all p's > 0.05). Thus, at this time, it is unclear what the role of ECS-kindling is in the intensi-

fication of the alcohol withdrawal syndrome.

These five experiments have clearly established that periodic ECSs can produce kindling-like effects, and that these periodic ECSs can intensify the alcohol withdrawal syndrome. However, the mechanisms underlying these effects are not yet fully understood. The results of these five experiments do provide guidelines for further investigations of the kindling effect and the interaction of periodic ECSs with other "convulsive" agents.

Reference List

Abrams, R. Multiple ECT: what have we learned? In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Abrams, R., & Fink, M. Clinical experiences with multiple electroconvulsive treatments. Comp. Psychiat., 1972, 13, 115-121.

Blumenthal, I.J. Spontaneous seizures and related electroencephalographic findings following shock therapy. J. Nerv. Ment. Dis., 1955, 122, 581-588.

Ervin, F.R., Mark, V.H., & Stevens, J.R. Behavioral and affective responses to brain stimulation in man. Proc. Amer. Psychopath. Assoc., 1969, 58, 54.

Essig, C.F., & Flanary, H.G. The importance of convulsion in occurrence and development of electroconvulsive threshold elevation. Exp. Neurol., 1966, 14, 448-452.

Essig, C.F., Groce, M.E., & Williamson, E.L. Reversible elevation of electroconvulsive threshold and occurrence of spontaneous convulsions upon repeated electrical stimulation of the cat brain. Exp. Neurol., 1961, 4, 37-47.

Fink, M. Clinical progress in convulsive therapy. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Folksman, A. Status epilepticus after electric convulsion therapy. Br. J. Med., 1947, 2, 335.

Frankel, F.H. Electroconvulsive therapy in Massachusetts: a task force report. Mass. J. Ment. Health, 1973, 3(2), 3-29.

Gallagher, B.B. Seizure threshold and hexafluorodiethyl ether in brain tissue. Biochem. Pharmacol., 1969, 18, 542-544.

Goddard. G.V. Development of epileptic seizures through brain stimulation at low intensities. Nature, 1967, 214, 1020-1021.

Goddard, G.V., McIntyre, D.C., & Leech, C.K. A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol., 1969, 25, 295-329.

Goodman, L.S., & Gilman, A., The pharmacological basis of therapeutics. Toronto: MacMillan Co., 1969.

Herberg, L.J., Tress, H.K., & Blundell, J.E. Raising the threshold in experimental epilepsy by hypothalamic and septal stimulations and by audiogenic seizures. Brain, 1969, 92, 313-328.

Holmberg, G. Effect on electrically-induced convulsions of the number of previous treatments in a series. Arch. Neurol. Psychiat., 1954, 71, 619-623.

Kalinowsky, L.B., & Hippius, H. Pharmacological, convulsive and other somatic treatments in psychiatry. New York: Grune & Stratton, 1969.

Karczmar, A.G. Brain acetylcholine and seizures. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Kety, S. Effects of repeated electroconvulsive shocks on brain catecholamines. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Leech, C.K. Sound-induced kindling resulting from daily bursts of loud noise presented to seven strains of mouse. Paper presented at the Canadian Psychological Association, June 3, 1971, St. John's, Newfoundland.

Majchrowicz, E. Induction of physical dependence on alcohol and associated metabolic and behavioral changes in the rat. Pharmacologist, 1973, 15, 159.

Mason, C.R., & Cooper, R.M. A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazol. Epilepsia, 1972, 13, 663-674.

McGaugh, J.L. Electroconvulsive shock: effects on learning and memory in animals. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Morrell, F. Goddard's kindling phenomenon: a new model of the "mirror focus". In H.C. Sabelli (Ed.), Chemical modulation of brain function. New York: Raven Press, 1973.

Morrell, F., & Tsuru, . Kindling in the frog: development of spontaneous epileptiform activity. EEG. Clin. Neurophysiol., 1975, in press.

Mucha, R.F., Pinel, J.P.J., & Phillips, A.G. Blockage of kindled seizures by antecedent stimulation to the focus. Paper presented at the Canadian Psychological Association, 1974, Windsor, Ontario.

Mucha, R.F., Pinel, J.P.J., & Van Oot, P.H. Simple method for producing an alcohol withdrawal syndrome in rats. Physiol. Biochem. Behav., 1975, In press.

Ottoson, J. Experimental studies of the mode of action of electroconvulsive shock. Acta. Psychiat. Neurol. Scand., 1960, Suppl. 145, 1-141.

Pacella, B.L., & Barrera, S.E. Spontaneous convulsions following convulsive shock therapy. Am. J. Psychiat., 1945, 101, 783-788.

Pinel, J.P.J., & Jones, R. Effects of antecedent footshock and current intensity on ECS-produced motor seizures in rats. Physiol. Psychol., 1973, 1, 241-244.

Pinel, J.P.J., Mucha, R.F., & Phillips, A.G. Spontaneous seizures generated in rats by kindling: a preliminary report. Physiol. Psychol., 1975, 3, 127-129.

Pinel, J.P.J., Phillips, A.G., & Deol, G.S. Effects of current intensity on kindled motor seizure activity in rats. Behav. Biol., 1974, 11, 59-68.

Pinel, J.P.J., Skelton, R., & Mucha, R.F. Effects of current intensity on afterdischarge threshold during kindling. Paper presented at Canadian Psychological Association, June, 1975, Quebec City, Quebec.

Pinel, J.P.J., & Van Oot, P.H. Generality of the kindling phenomenon: some clinical implications. Can. J. Neurol. Sci., 1975, Nov., 467-475.

Pinel, J.P.J., Van Oot, P.H., & Mucha, R.F. Intensification of the alcohol withdrawal syndrome by prior repeated brain stimulation. Nature, 1975, 254, 510-512.

Pollack, M., Rosenthal, F., & Macey, R. Changes in electroshock convulsive response with repeated seizures. Exp. Neurol., 1963, 7, 98-106.

Post, R.M., & Kopanda, R.T. Cocaine, kindling and reverse tolerance. Lancet, 1975, 15, 409-410.

Post, R.M., Kopanda, R.T., & Black, K. Progressive effects of cocaine on behavior and central amine metabolism in rhesus monkeys. Biol. Psychiat., 1975, In press.

Post, R.M., Kopanda, R.T., & Lee, A., Progressive behavioral changes during chronic lidocaine administration: relationship to kindling. Life Sci., 1975, 17, 943-950.

Prichard, J.W., Gallagher, B.B., & Glaser, G.H. Experimental seizure-threshold testing with fluorothyl. J. Pharmac. Exp. Ther., 1969, 166, 170-178.

Pryor, G.T. Effect of repeated ECS on brain weight and brain enzymes. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Pryor, G.T., & Otis, L.S. Persisting effects of chronic electroshock seizures on brain and behavior in two strains of rats. Physiol. Behav., 1970, 5, 1053-1055.

Racine, R. Modification of seizure activity by electrical stimulation: I afterdischarge threshold. EEG. Clin. Neurophysiol., 1972a, 32, 281-294.

Racine, R. Modification of seizure activity by electrical stimulation: II motor seizures. EEG. Clin. Neurophysiol., 1972b, 32, 281-294.

Racine, R. Modification of seizure activity by electrical stimulation: cortical areas. EEG. Clin. Neurophysiol., 1975, 38, 1-12.

Racine, R. Burnham, W.M., Gartner, J.G., & Levitan, D. Rates of motor seizure development in rats subjected to electrical brain stimulation: strain and interstimulation interval effects. EEG. Clin. Neurophysiol., 1973, 35, 553-556.

Ramer, D., & Pinel, J.P.J. Kindling effect and ECS-induced seizures in rats. Paper presented at the Canadian Psychological Association, June, 1974, Windsor, Ontario.

Rovner, L.I., & Pinel, J.P.J. Kindling of spontaneous clinical seizures in rats. Paper presented at the Canadian Psychological Association, June, 1976, Toronto, Ontario.

Scheffe, H. The analysis of variance. New York: J. Wiley & Sons, 1959.

Schildkraut, J.J., & Draskoczy, P.R. Effects of electroconvulsive shock on norepinephrine turnover and metabolism: basic and clinical studies. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Small, J.G. EEG and neurophysiological studies of convulsive therapies. In M. Fink, S. Kety, J.L. McGaugh, & T.A. Williams (Eds.), Psychobiology of convulsive therapy. Washington, D.C.: V.H. Winston & Sons, 1974.

Stevens, J.R., Mark, V.H., Erwin, F., Pacheco, P., & Suematsu, K. Deep temporal stimulation in man, long latency, long lasting psychological changes. Arch. Neurol., 1969, 21, 157-169.

Tanaka, A. Progressive changes of behavioral and electroencephalographic responses to daily amygdaloid stimulations in rabbits. Fukuoka Acts. Medica., 1972, 63, 152-164.

T-W-Fiennes, R.W., Harrison, F.A., Ray, P., & Scott, W.N. (Eds.), The UFAW handbook on the care and management of laboratory animals. London: Churchill Livingston Ltd., 1972.

Vosu, H., & Wise, R.A. Cholinergic seizure kindling in the rat: comparison of caudate, amygdala, and hippocampus. Behav. Biol., 1975, 13,

Wada, J.A., Osawa, T., & Mizoguichi, T. Recurrent spontaneous seizure state induced by prefrontal kindling in Senegalese baboons, Papio Papio. Can. J. Neurol. Sci., 1975, 2, 477-492.

Wada, J.A., & Sato, M. Recurrent spontaneous epileptic seizure state induced by localized electrical stimulation. Neurology, 1973, 23, 447.

Wada, J.A., Sato, M., & Corcoran, M.E. Persistent seizure susceptibility and recurrent spontaneous seizures in kindled cats. Epilepsia, 1974, 15, 465-478.

Woodbury, M.R. Mechanisms of action of anticonvulsants. In H. Jasper, A. Ward, & A. Pope (Eds.), Basic mechanisms of the epilepsies. Boston: Little, Brown, & Co., 1969.

Woodbury, M.R., & Davenport, V.D. Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. Arch. Int. Pharmacodyn., 1952, 92, 97-107.

Zarrow, M.X., Pawlowski, A.A., & Denenberg, V.H. Electroshock convulsion threshold and organ weights in rats after alcohol consumption. Am. J. Physiol., 1962, 203, 197-200.

Appendix I

Mean Values for the Alcohol-tolerance Measures for each Group receiving Alcohol in each Experiment

Group[a]	Initial Tolerance	Final Tolerance	Change in Tolerance
	Experiment 1		
Control (12)	1.80	2.80	0.97
Pseudo-ECS (9)	1.90	2.70	0.80
15-mA ECS (11)	2.20	3.10	0.84
75-mA ECS (13)	2.30	2.90	0.62
	Experiment 2		
Control (13)	2.10	3.20	1.15
3-day, ETOH (12)	2.10	3.30	1.22
1-hr, ETOH (10)	2.60	3.10	1.06
	Experiment 3		
Control (13)	2.10	3.20	1.15
20 ECSs (8)	2.30	3.20	0.98
10 ECSs (9)	2.20	3.20	0.96
6 ECSs (9)	1.90	3.10	1.02
3 ECSs (9)	2.00	3.00	1.04
	Experiment 4		
Control (13)	2.08	3.23	1.15
2-week delay (9)	2.20	3.16	0.96
3-week delay (10)	2.36	3.44	0.78
6-week delay (10)	2.14	3.22	1.08
10-week delay (7)	1.94	2.97	1.03
	Experiment 5		
Control (9)	2.22	2.89	0.67
ECS (12)	1.90	3.05	1.15
ECS + Drugs (10)	2.23	3.21	0.90
Drugs (8)	2.18	3.08	0.90

Note. Values are expressed in grams/kilogram.

[a]Numbers in parentheses are the number of animals per group.

Appendix II

Table of Scheffe Values for the Individual, Between-group Comparisons of the Tolerance-related Measures of Experiment I

Group	Pseudo-ECS	15-mA ECS	75-mA ECS
		Initial Tolerance[a]	
Control	(1,19) = 2.17	(1,21) = 17.05[a]	(1,23) = 19.22[a]
Pseudo-ECS		(1,18) = 4.65	(1,20) = 6.83
15-mA ECS			(1,22) = 0.52
75-mA ECS			
		Final Tolerance[a]	
Control	(1,19) = 0.20	(1,21) = 15.76[a]	(1,23) = 4.33
Pseudo-ECS		(1,18) = 16.21[a]	(1,20) = 4.91
15-mA ECS			(1,22) = 1.77
		Change in Tolerance[a]	
Control	(1,19) = 2.87	(1,21) = 1.74	(1,23) = 14.49[a]
Pseudo-ECS		(1,18) = 0.11	(1,20) = 3.64
15-mA ECS			(1,22) = 5.24

Note. Numbers in parentheses indicate the degrees of freedom

[a] $p < 0.01$.