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Alcohol Withdrawal Syndrome in Rats: Neural and Behavioral Correlates[1]

BRUCE E. HUNTER, CARL A. BOAST, DON W. WALKER AND STEVEN F. ZORNETZER

Department of Neuroscience, College of Medicine
University of Florida and Veterans Administration Hospital
Gainesville, Florida 32610

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HUNTER, B. E., BOAST, C. A., WALKER, D. W. AND S. F. ZORNETZER. *Alcohol withdrawal syndrome in rats: neural and behavioral correlates.* PHARMAC. BIOCHEM. BEHAV. 1(6) 719–725, 1973.–Rats were chronically implanted with electrodes in the ventral hippocampus, amygdala and anterior cortex and maintained on liquid diets as their only source of calories and fluid for 15 days. The diet consisted of 35–40% of the calories in the form of ethanol while a control group was pair-fed identical diets with sucrose isocalorically substituted for ethanol. On the sixteenth day the diets were removed and electrographic activity and behavior were simultaneously observed for 8–10 hr. Withdrawal symptoms were observed beginning 2–4 hr following alcohol abstinence and included tail-stiffening, tremors, severe ataxia and auditory-induced convulsions. EEG epileptiform activity was observed and initially consisted of transient spike events, which usually became progressively organized into brief spike bursts or sustained paroxysmal activity. The results suggested that cortical bioelectric activity may not play a primary role in the genesis of behavioral hyperexcitability during alcohol withdrawal. The utility of the method of combined observations of neural bioelectric activity and behavior for the delineation of the neural substrates of alcohol withdrawal symptoms was discussed.

Alcohol Drug dependence Alcohol withdrawal syndrome EEG

THE STUDY of alcoholism has been hampered by an inability to develop an animal model which faithfully approximates the psychological and physiological characteristics manifested in the human condition. Several criteria have been established for such a model [19]. Perhaps the most problematic of these criteria is the voluntary selection of intoxicating quantities of ethanol since most animals exhibit a strong initial aversion to this substance [24]. Although the factors which contribute to increases in the preference for ethanol as a function of the development of physical dependence remain obscure, several pharmacological models [22] have been developed which provide a method to study the behavioral and physiological consequences of prolonged ethanol consumption. The technique common to each of these models is the forced consumption of ethanol by a variety of means including oral [9, 10, 25], intravenous [4,30], intragastric [5, 8, 12] and respiratory [14] routes of administration. The emphasis in these studies has been on the development of techniques to induce physical dependence and the development of valuable behavioral criteria for evaluation of the intensity of the withdrawal syndrome [10,13].

The behavioral symptoms observed during alcohol withdrawal are believed to reflect the release of a latent state of "neural hyperexcitability" [18,23] proposed to result from a variety of cellular adaptive mechanisms [3, 17, 20]. The presence of this neural hyperexcitability has been largely inferred from behavioral observations derived from techniques such as threshold reduction for startle responses [12] or convulsions elicited by electroconvulsive shock [21], chemical [26] or auditory stimulation [11,26]. To date, little is known about the neural mechanisms underlying the development of hyperexcitability during alcohol withdrawal. One technique that has not been effectively utilized in animal research concerned with the withdrawal syndrome is electrophysiological monitoring of specific brain regions.

There is an extensive clinical literature concerned with electroencephalographic (EEG) abnormalities recorded from human alcoholics [1]. Few studies have dealt with the alcohol withdrawal syndrome [1], possibly due to inherent methodological difficulties. For example, tremors and general agitation during withdrawal make it difficult to obtain sufficient duration of artifact-free scalp EEG [29]. Further, routine medication during alcohol withdrawal obscures the interpretation of EEG recordings [1]. Finally EEG dysrhythmias during alcohol withdrawal in humans have been characterized as mild [29]. However, since human EEG recordings consist almost exclusively of scalp recordings, substantial abnormal subcortical bioelectric activity may be present but undetected.

The present experiment was designed to (1) develop a

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reliable model of alcohol withdrawal in rats using the liquid diet technique previously used for mice by Freund [10] and, (2) to attempt to characterize the EEG abnormalities recorded directly from deep and surface brain regions during the withdrawal syndrome.

METHOD

Animals

Twenty-seven male, hooded rats weighing 250–350 g were used in the experiment. They were housed individually in stainless steel cages in a colony room having a 7:00 a.m. to 7:00 p.m. light cycle. Food and water were available ad lib prior to and during recovery from surgery.

Surgery

Stereotaxic surgery was performed under Nembutal anesthesia (50 mg/kg). Three monopolar depth electrodes, one bipolar electrode and two monopolar cortical screw electrodes were implanted in each animal. The monopolar depth electrodes (00 stainless steel insect pins insulated with epoxylite to within 0.5 mm of the tip) were implanted in the right amygdala and bilaterally in the ventral hippocampus. A twisted bipolar platinum-iridium electrode (125 μ) was implanted in the left amygdala. Coordinates, relative to bregma, for the amygdala were: 0.5 mm posterior of bregma, 4.7 mm on either side of midline, and 9.0 mm from brain surface. Coordinates for the hippocampus were: 3.6 mm posterior of bregma, 5.5 mm on either side of midline, and 8.3 mm from brain surface. The two stainless steel screws (1/8 in. × 080) were placed in the skull overlying anterior cortex, 3 mm anterior of bregma and 3 mm on either side of midline. A similar screw placed in the frontal sinus served as ground. Electrodes were soldered to a nine pin ITT Cannon connector and the entire assembly was fixed to the skull with dental cement.

Liquid Diets

Details of the preparation, composition and nutritional adequacy of the liquid diets used in this experiment have been presented previously [27]. Briefly, the diet was prepared from a 63.3% (v/v) stock solution (prepared from 95% ethanol and distilled water) mixed with Metrecal Shape (Mead Johnson Co.) and contained 35–40% ethanol-derived calories. The concentration of ethanol ranged from 8.1–9.7% (v/v) with the diet providing approximately 1.3 Kcal/ml. Sucrose was isocalorically substitued for ethanol in control diets. Both the alcohol and sucrose diets were additionally fortified with Vitamin Diet Fortification Mixture, 0.3 g/100 ml of diet, and Salt Mixture XIV, 0.5 g/100 ml of diet (Nutritional Biochemicals Corporation). The diets were prepared fresh daily and administered in calibrated bottles.

Procedure

Following a 9–16 day recovery period the rats were divided into three groups (nine each) matched for body weight and reduced to 75% of their free feeding weight. Two of the groups (alcohol withdrawal, AW; and alcohol control, AC) then received the alcohol liquid diet and the remaining control group was pair-fed the sucrose liquid diet (SC). The lower quantity of liquid diet consumed by either an AW or AC rat, was pair-fed to each corresponding matched SC control.

All rats were maintained on their respective diets for 15 days. The percent of total calories as ethanol in the liquid diets was gradually increased from 35–40% during this period. Two AW rats were sacrificed after their head plug assemblies became disconnected during the 15-day treatment period. On Day 16 the diets were removed from the AW and SC groups. Each AW rat was connected to a flexible, low noise, shielded cable (Microdot, Inc.) and placed in a recording chamber located in a shielded room. The recording chamber (12 × 18 × 18 in.) had a Plexiglas observation window. EEG activity from all electrode placements was continuously monitored on a Grass Model 7 polygraph. Behavior was simultaneously observed for 8–10 hr postwithdrawal..

During the 8–10 hr period AC and SC rats were also observed and sample periods of EEG activity recorded. The withdrawal schedule of the rats was staggered such that only one AW rat (with its corresponding SC and AC controls) was withdrawn on a given day. During the alcohol consumption period one AW rat dislodged its diet bottle and presumably underwent withdrawal during the night prior to its scheduled withdrawal. Therefore a total of 6 AW rats was observed during the scheduled withdrawal period.

On the day following withdrawal, the AW rats were replaced on the alcohol diets as part of a separate chronic experiment. After 15 days of continued alcohol consumption, control EEG recordings were obtained from these rats.

Histology

Following the experiment all rats were given an overdose of Nembutal and were intracardially perfused with 0.9% saline followed by 10% formalin. The brains were removed, embedded in celloidin, and sectioned at 30 microns. Sections through the electrode tracks were stained with cresyl violet. Histological verification of amygdaloid and hippocampal electrode tip locations was obtained in 3 AW rats. All cortical placements were verified.

RESULTS

The mean free feeding weights for the AW, AC, and SC groups were 339, 330 and 324 g respectively. No weight loss was observed during the liquid diet treatment period nor was there any evidence of malnutrition. All rats gained weight during the liquid diet treatment period, with the mean weights returning from 75% (Day 1) to 90–95% of their original predeprivation values on the day of withdrawal (Day 16). Ethanol consumption during the 15-day treatment period was uniformly high. Mean daily consumption expressed as g/kg/rat/day was 15.2 (range: 14.0–16.4) for the AW group and 16.0 (range: 14.7–17.3) for the AC group. Daily observations of AW and AC rats revealed signs of gross intoxication including ataxia, docility and loss of coordination.

The time course and behavioral symptoms observed during the alcohol withdrawal period were strikingly similar to those reported previously in mice [10]. Initially there was a period of motor hyperactivity together with the development of piloerection and tail stiffening 2–4 hr postwithdrawal. This hyperactivity was usually transient and was followed by gross hypoactivity which persisted for the remainder of the observation period. Hypoactivity developed and progressed, commensurate with the onset of the most severe withdrawal symptoms including tremors

and muscular fasciculations, severe ataxia, rigidity, hyperreactivity and often, but more variably, spontaneous vocalizations. The development of behavioral symptoms was similar in all AW rats. These symptoms usually developed gradually and were observed to be most servere 6–10 hr postwithdrawal. At the point where the behavioral symptoms no longer appeared to be increasing in severity the rats were tested for susceptibility to auditory-induced convulsions. A shaking of keys (3–8 sec) near the top of the recording chamber resulted in running episodes and tonic–clonic convulsions with a duration ranging from 30 60 sec in all 6 AW rats. Some convulsions were characterized by the rats leaping out of the recording chamber, emitting spontaneous vocalizations, and exhibiting hyperreactivity and aggressiveness when attempts were made to return them to the recording chamber. The abovementioned constellation of behavioral symptoms was never observed in either SC rats, which were also withdrawn from their diets, or AC rats which had continued access to ethanol. Furthermore auditory stimulation in AC and SC rats (shortly following elicitation of convulsions in AW rats) had no effect.

EEG recording from AW rats during the withdrawal period indicated the widespread development of abnormal cortical epileptiform activity. The abnormalities usually began with the development of synchronous activity (1 5 Hz) together with transient spikes resembling interictal epileptiform events. The appearance of transient spiking roughly coincided with the initial appearance of behavioral symptoms. The epileptiform spike events increased, both in amplitude and frequency, with a tendency to become more organized during the latter stages of withdrawal (4 6 hr). This increased organization consisted of brief bursts of spike activity or sustained epileptiform episodes.

Cortical EEG activity during withdrawal was continously evaluated up to the time auditory-induced convulsions were elicited and classified into one of the following four stages: *Stage I* – Synchronized high amplitude EEG together with transient spike events (a peak to peak amplitude of at least twice background activity was judged a spike event) occurring with a frequency of less than one/min; *Stage II* – Increased occurrence and amplitude of spike events with a frequency of from 1 10/min; *Stage III* – Organized bursts of spike activity consisting of an envelope of 3 10 spikes within a 3 5 sec epoch; or *Stage IV* – Sustained seizure-like activity.

The results of this analysis for 5 AW rats are shown in Table 1. Differences in the degree and development of cortical epileptiform activity could not be attributed to variability in the severity of behavioral symptoms or the level of ethanol consumption (see bottom Table 1) during the 15-day treatment period. Table 1 indicates that although the final stage of severity differed among AW animals, there nevertheless was a progressive development of organized cortical epileptiform activity during the withdrawal period. The EEG abnormalities appeared to progress in a correlated fashion with the behavioral symptoms. However, EEG epileptiform activity did not appear to be causative of specific behavioral abnormalities. Thus, for example, a specific tremor or behavioral automatism did not necessarily coincide with a spike event or other EEG anomaly. One AW rat was not included in Table 1, due to lack of experimental control over the withdrawal period. This rat was observed to have severe

TABLE 1

ANALYSIS OF CORTICAL EEG ACTIVITY DURING ALCOHOL WITHDRAWAL* AND INDIVIDUAL ALCOHOL CONSUMPTION

Hours Postwithdrawal	AW 25	AW 21	AW 15	AW 8	AW 20
1.	–			–	
2.		II			I
3.	II	II	I		I
4.	III	II	I	–	I
5.	III	III	I		I
6.	IV	III	III	I	I
7.			III	I	
8.				III	
9.				III	
10.				III	
Alcohol Consumption†	14.7	13.1	15.8	17.0	13.0

*See text for details of Stage I, II, III and IV.

†G/KG/DAY during the last five days of the Alcohol Treatment period.

behavioral symptoms during routine morning maintenance. Further investigation indicated that the tube on its liquid diet bottle had become clogged with a membrane of dried diet. EEG recordings revealed frequent high amplitude spiking (Stage II) and auditory stimulation resulted in a tonic- clonic convulsion.

Figure 1 shows sample cortical EEG recordings from rat AW No. 25 during the progressive development of epileptiform activity 3, 4, 5 and 6 hr postwithdrawal. Frequent high amplitude spiking (Fig. 1A) developed 3 hr postwithdrawal and progressed in severity during the fourth hour. Note that although brief bursts of spiking persisted during hours 4 5, their appearance changed substantially as can be seen by comparing Fig. 1B and 1C. Sustained paroxysmal activity occurred prior to the elicitation of a convulsion (Fig. 1D). Figure 2 shows a somewhat different developmental pattern of cortical epileptiform activity recorded in AW No. 21 (Fig. 2A) and AW No. 15 (Fig. 2B). In both cases the most severe behavioral symptoms were observed, and auditory-induced convulsions elicited, while cortical EEG activity was judged to be Stage III.

Amygdaloid and hippocampal recordings in AW rats in which histological verification was obtained, indicated a similar temporal development of epileptiform activity as described above for cortical EEG. It is important to note, however, that although these three subcortical brain regions exhibited abnormal EEG activity, there was substantial independence among these structures with respect to the occurrence of specific spike events. These results suggest that widespread areas of forebrain develop similar patterns of paroxysmal activity during alcohol withdrawal.

EEG recordings during auditory-induced convulsions were obtained in 3 AW rats. As mentioned previously, these convulsions were violent, accounting for the loss of EEG

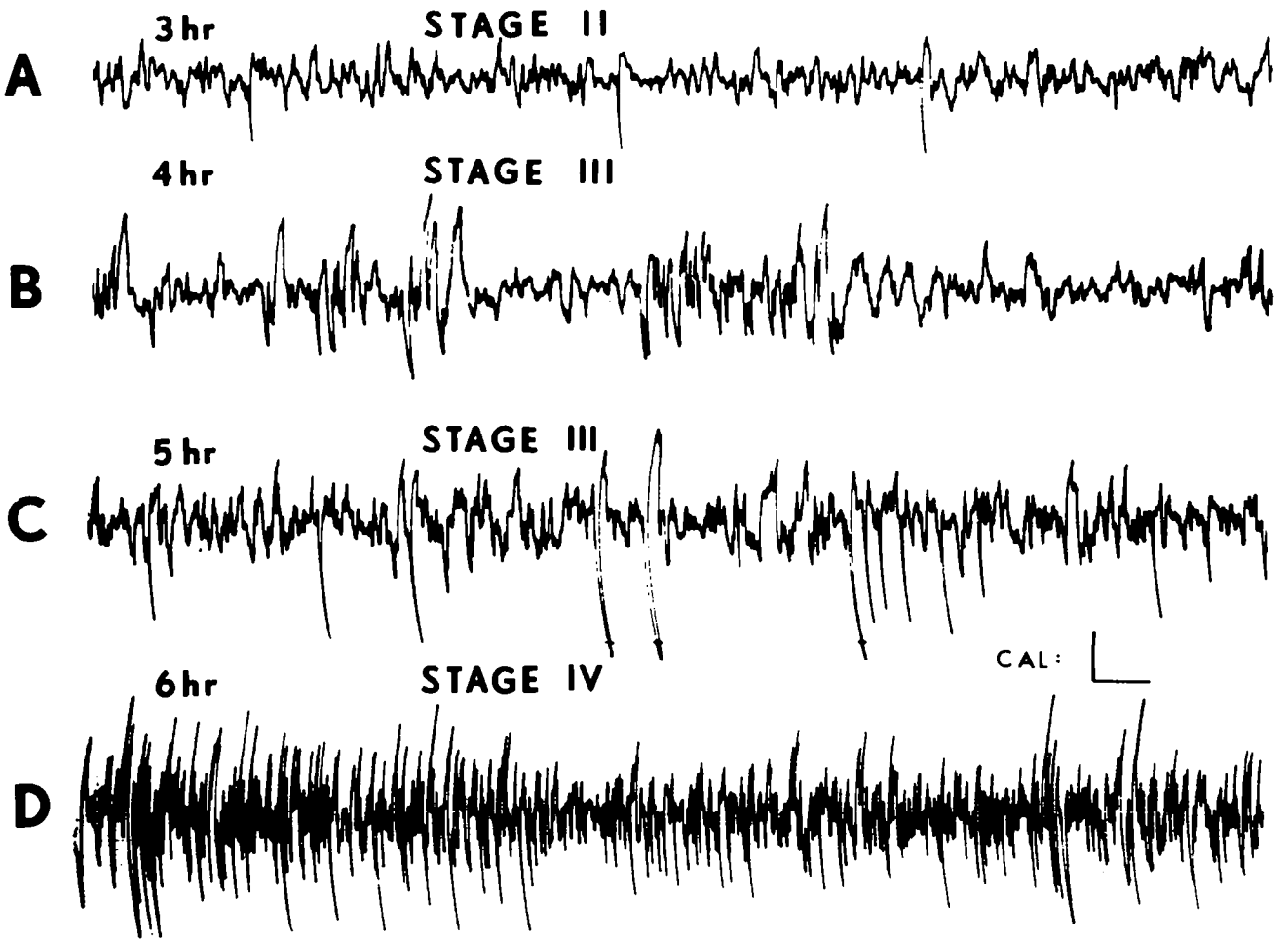

FIG. 1. Sample EEG recordings from anterior cortex in AW No. 25. (A) Stage II spiking 3 hr postwithdrawal. Calibration: 100 μV, 1 sec. (B) and (C) Stage III spike bursts 4 and 5 hr postwithdrawal. Calibration: 100 μV, 1 sec. (D) Stage IV seizure activity 6 hr postwithdrawal. Calibration 100 μV, 2 sec.

data from the remaining AW animals which became detached from the recording cable during their convulsions. Figure 3 shows the patterns of forebrain EEG activity recorded during auditory-induced convulsions. As Fig. 3A indicates, sustained seizure activity did not occur in anterior cortex despite the occurrence of prolonged tonic–clonic convulsions. In two rats, hippocampal seizure activity was observed, but with a significant delay after the onset of the convulsion. In the third rat, no forebrain seizure activity was recorded during the convulsion. In Fig. 3A only isolated spiking was recorded in cortex throughout the extent of the convulsion (onset indicated by arrow). Sustained seizure activity began in the hippocampus approximately 30 sec after the onset of the convulsion (Fig., 3B). Note also the appearance of a period of secondary seizure discharge after the initial seizure subsided. The observed dissociation between forbrain EEG and behavioral convulsions suggests that seizure activity need not spread over the entire brain for a convulsion to occur and more importantly, that epileptiform activity observed in forebrain and associated with convulsions may have been propagated from distant brain loci. Control recordings during auditory stimulation in AC and SC rats, as well as in AW rats 15 days after alcohol diets had been reestablished, resulted in neither convulsions nor evidence of forebrain epileptiform activity.

DISCUSSION

The time course of alcohol withdrawal and the specific behavioral symptomatology observed in the present experiment is consistent with previous reports in rodents [10]. Although ethanol dependence, as indicated by withdrawal symptoms, has been experimentally induced in a variety of species [5, 8, 10], as well as in man [29], only recently has physical dependence in rats been observed [2,9]. Falk, *et al.* [9] observed auditory-induced convulsions in two rats following ethanol abstinence after nearly 4 months of consumption using a modified schedule-induced polydipsia technique. The results of the present experiment, including level of ethanol consumption, rapid weight gain, observations of gross intoxication and behavioral symptoms of physical dependence following withdrawal, together with the results of another experiment using a somewhat similar

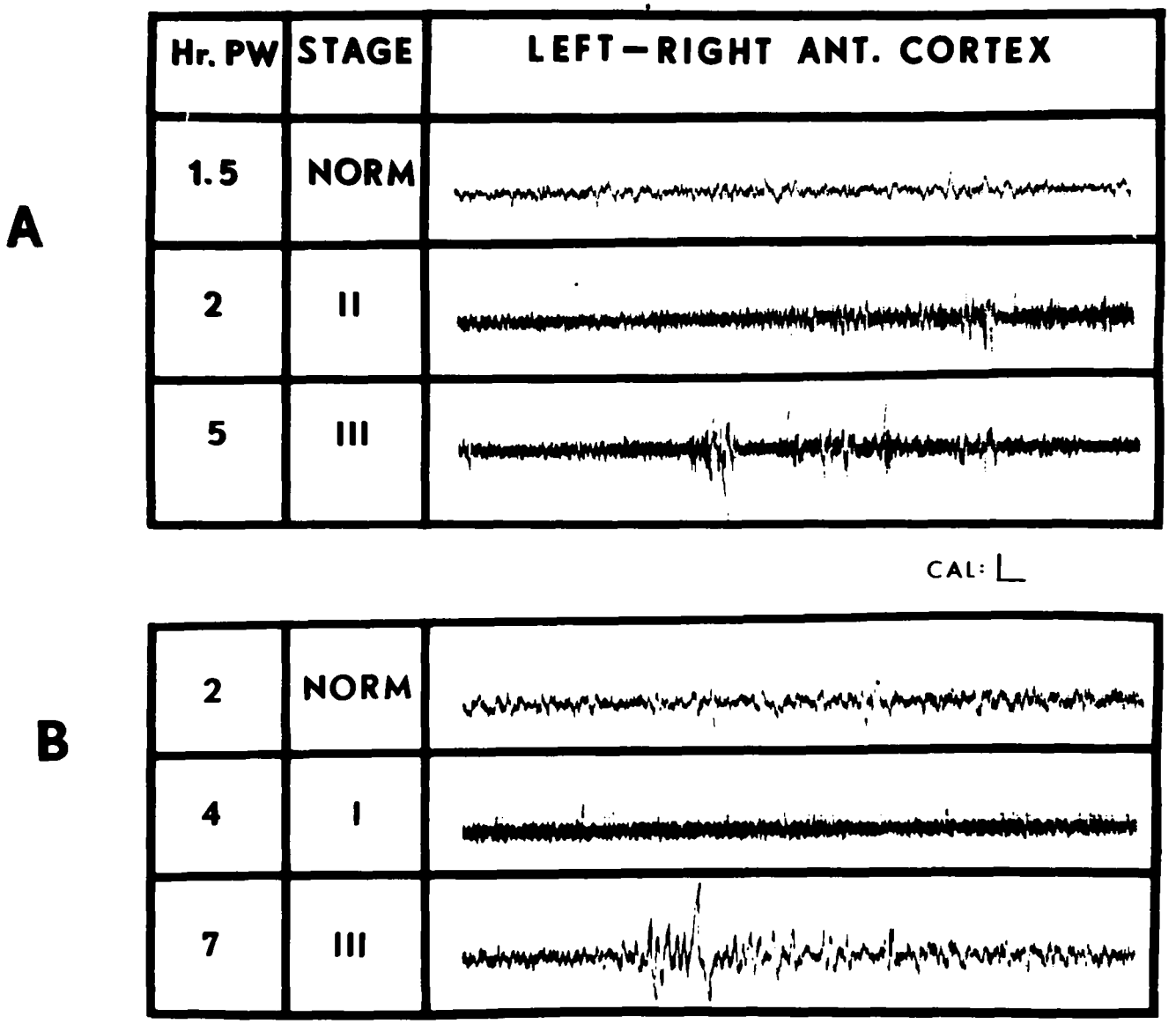

FIG. 2. (2A) Stages of cortical EEG activity in AW No. 21 during alcohol withdrawal. Calibration: 1.5 hr postwithdrawal (P.W.), 100 μV, 1 sec; 2 hr P.W., 100 μV, 4 sec, 5 hr P.W., 100 μV, 4 sec. (2B) Stages of cortical EEG in A. W. No. 15. Calibration: 2 hr P.W., 100 μV, 1 sec; 4 hr P.W., 100 μV, 4 sec; 7 hr P.W., 100 μV, 1 sec.

procedure [2], suggests that physical dependence can be reliably produced in rats after 2–3 weeks of ethanol consumption using the liquid diet technique.

The results of the present experiment further indicate a progressive development of forebrain epileptiform activity during the first few hours following ethanol abstinence. EEG abnormalities characteristically began with slowing, coupled with the appearance of transient spiking. The spiking usually progressed in severity and became organized into bursts of spike events or sustained seizure activity. A similar progression of epileptiform activity recorded from a variety of forebrain areas has recently been reported in mice [28]. Furthermore, Guerrero-Figueroa, *et al.* [16] have observed paroxysmal abnormalities in cortical and subcortical areas in cats who had received alcohol via gastric fistulae for 2–5 months. These data [16] are difficult to interpret, however, since no distinctions were made between EEG activity observed during the withdrawal period in cats with chronic, chemically-induced epileptiform foci, and normal cats.

This forebrain epileptiform activity observed during alcohol withdrawal suggests two interpretations: (1) independent loci of neural hyperexcitability develop throughout forebrain or, (2) a structure or set of structures serve to organize and propagate epileptiform activity to forebrain areas. The present results could be interpreted to support both of these suggestions. Thus, epileptiform activity appeared to develop independently in anterior cortex, ventral hippocampus, and anygdala during the early hours following alcohol withdrawal. However, forebrain

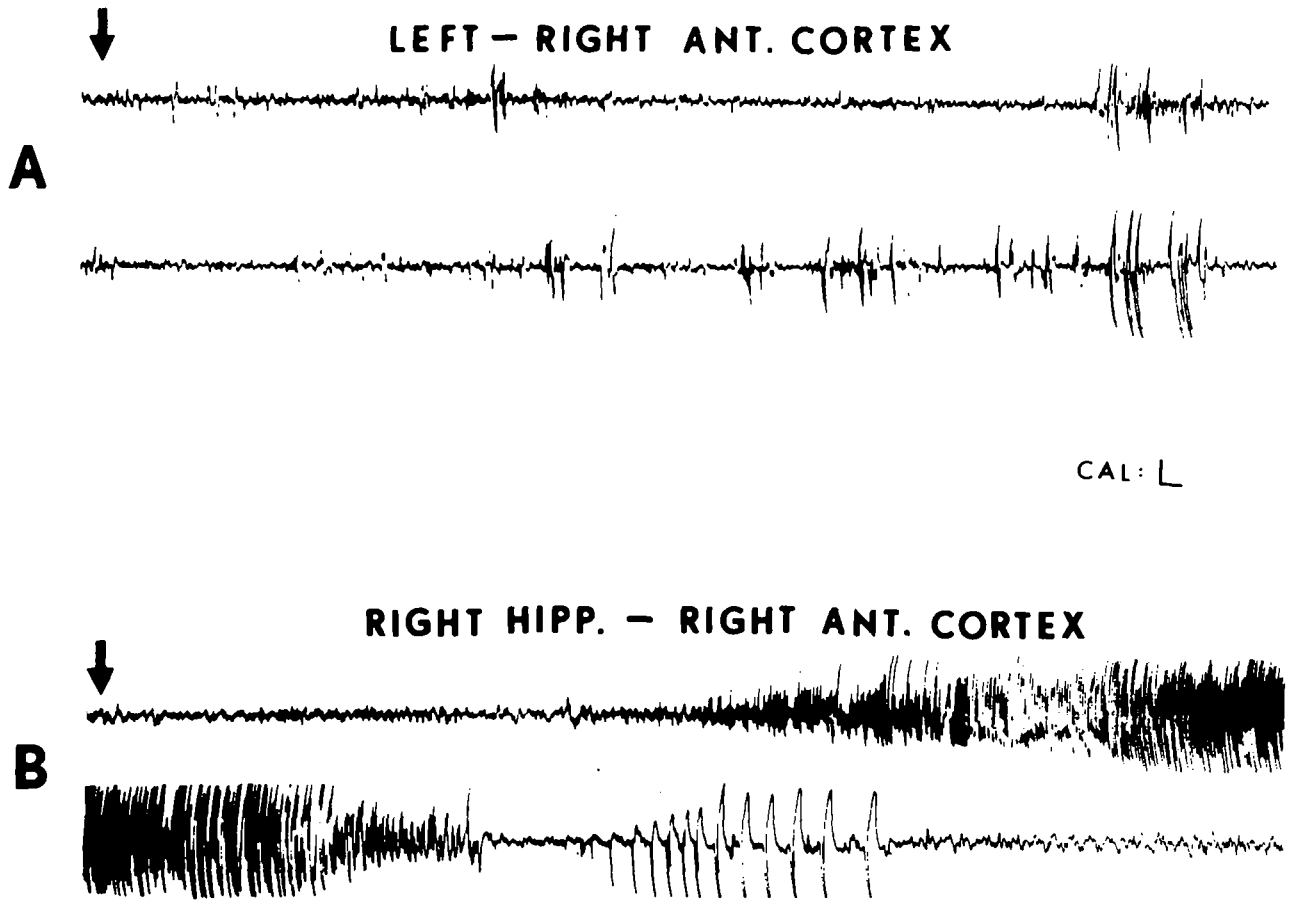

FIG. 3. Simultaneous EEG activity from cortex and hippocampus during extent of auditory-induced convulsion (onset indicated by arrow). (A) EEG activity from cortex indicating only isolated spiking during convulsion. Calibration: 100 μV, 1 sec. (B) Hippocampal EEG activity during convulsions showing sustained seizure beginning 30 sec after onset of convulsion. Calibration: 300 μV, 1 sec. In both 2A and B bottom traces are continuation of top traces.

seizure activity during auditory-induced convulsions appeared to be propagated from some distant brain loci, reaching forebrain with a significant delay after the onset of the convulsion. These results indicate the possibility that the above suggestions may not be mutually exclusive. Thus, for example as alcohol concentration dissipates throughout brain following withdrawal, epileptiform activity may develop in an independent fashion in many brain regions. The most severe behavioral symptoms, especially convulsions, would be contingent upon the influence of some structures which serve to organize the epileptiform events into sustained patterns of seizure activity.

The results of the present experiment suggest that cortical epileptiform activity per se may play a secondary role in the genesis of behavioral hyperexcitability during alcohol withdrawal. If hyperexcitability during withdrawal were primarily contingent upon cortical bioelectric activity it would be expected that, (1) specific behavioral automatisms would occur coincident with specific bioelectric events, (2) more intense focal epileptiform activity would develop in cortex when compared to other brain regions, (3) the level of cortical EEG abnormalities would correlate with the intensity of withdrawal symptoms, and (4) cortical seizure activity would be closely associated with behavioral convulsions. In the present experiment it was found that no specific behavioral concomitants of cortical epileptiform events could be observed. Previously, convulsions induced by auditory stimulations have been used as an index of behavioral hyperexcitability during alcohol withdrawal [11,26]. In the present experiment convulsions were elicited while cortical epileptiform activity was judged to be Stage I (n = 1), II (n = 1), III (n = 3), or IV (n = 1), indicating that cortical EEG activity does not provide an index of the level of behavioral hyperexcitability. Further, the cortex, amygdala and hippocampus had a similar developmental pattern of EEG epileptiform activity. At no point did EEG abnormalities recorded from amygdala or hippocampus appear to be propagated from cortical areas. Finally, sustained seizure activity was not observed in cortex despite prolonged behavioral convulsions. These results must be considered indirect, but nevertheless, taken together, they support the conclusion that the cortex does not play a primary role in the genesis of behavioral hyperexcitability during alcohol withdrawal.

This conclusion is also supported by evidence obtained during withdrawal from other depressant agents. For example, EEG recordings in cats have indicated that the cortex may also play a secondary role in the genesis of

behavioral symptoms during barbiturate withdrawal [6]. However, a substantial alteration of barbiturate withdrawal symptoms in decorticate dogs [7], indicates that an intact cortex is required for the manifestation of the normal pattern of observed withdrawal symptoms, but not a prerequisite for the induction of behavioral hyperexcitability during withdrawal from depressant agents.

The method reported here should prove useful in studying alterations in neural and behavioral excitability observed during alcohol withdrawal. Future studies, involving systematic observations of abnormal bioelectric activity from multiple recording sites should allow a more precise delineation of CNS areas involved in the genesis and propagation of neural epileptiform activity during alcohol withdrawal. In addition, combining various electrophysiological and behavioral techniques should enable a more direct evaluation of the effects of chronic ethanol consumption including the development of tolerance, physical dependence, and the alcohol-induced disruption of the processes of learning [27].

Since substantial controversy still exists over the appropriate medication for alcohol withdrawal [15], an animal model for the evaluation of potentially useful drugs is desirable. The dissociations between behavioral symptoms of alcohol withdrawal and forebrain epileptiform activity observed in the present experiment in rats, and also recently in mice [28], indicates that a combination of EEG and behavioral monitoring techniques may provide a more sensitive index of the severity of the withdrawal reaction than either technique alone. We believe that simultaneous monitoring of both EEG and behavior is desirable in the preclinical evaluation of therapeutic agents for the treatment of alcohol withdrawal. Finally, the liquid diet technique appears ideal for such an evaluation as well as for the investigation of neruophysiological, biochemical and behavioral correlates of the alcohol withdrawal syndrome since physical dependence can be rapidly developed, nutritional variables can be adequately controlled [27] and animal maintenance involves little effort relative to other models currently in use.

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