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Effects of Alcohol on Kindled Seizure Thresholds in Rats

FREDERICK G. FREEMAN

Department of Psychology, Old Dominion University, Norfolk, VA 23508

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FREEMAN, F. G. *Effects of alcohol on kindled seizure thresholds in rats.* PHARMAC. BIOCHEM. BEHAV. 8(6) 641–644, 1978. – Seizures were kindled in the amygdala and ventral hippocampus of rats until a stage 5 (clonic convulsion) was elicited. Stage 5 thresholds were then determined. Animals were then injected with either saline, or a 600 mg/kg, or 1600 mg/kg dose of 25% ethanol. The effect of each of these injections on seizure thresholds was assessed. The 1600 mg/kg dose caused a significant elevation in both AD and motor seizure thresholds, relative to the 600 mg/kg dose and saline, which did not differ. The elevation of seizure thresholds was significantly greater for animals with seizures kindled in the ventral hippocampus.

Seizure threshold Alcohol Limbic system

THE ADMINISTRATION of ethanol often has a depressant effect on convulsive thresholds. Both audiogenic seizures [5] and seizures induced by electrical stimulation [1, 8, 10, 11, 18, 19] have higher thresholds following alcohol administration. Although the effect of ethanol on artificially induced generalized seizures appears clear cut, relatively little research has been conducted investigating the responsiveness of selected nuclei to seizure inducing stimuli following the administration of ethanol. Caspers [3] observed an increase in convulsion-like activity and prolonged afterdischarges elicited by direct stimulation of the cortex of rats following low doses of ethanol. Baker and Benedict [2] observed a decrease in activity of chemically induced seizures in the hippocampus following alcohol administration. However, direct injection of alcohol into the hippocampus enhanced seizure like activity. Guerrero-Figueroa, Rye, Gallant, and Bishop [9] studied the effect of chronic ethanol administration of epileptogenic foci induced by cobalt or aluminum oxide in the hippocampus, amygdala, mesencephalic reticular formation and sensory-motor cortex of cats. During the first week of ethanol treatment, 80% of all seizures observed were in animals with amygdalar and hippocampal foci.

Other studies which did not involve artificially induced seizures also suggest a differential responsiveness to ethanol by the limbic system. High voltage potentials and spiking and afterdischarges following withdrawal from chronic alcohol administration have been observed in the hippocampus, septal area, thalamus, and cortex [14,18]. Finally, Perrin, Hockman, Kalant, and Livingston [13] observed that the depressant effect of ethanol recorded from various cortical and subcortical areas was not observed in the ventral hippocampus.

In the present study the effect of ethanol on seizures kindled in the amygdala and hippocampus was studied. In order to maintain relatively precise control over the seizure inducing stimuli, an experimental model of epilepsy, kindling, was used. Kindling was first investigated systematically by Goddard, McIntyre, and Leech [7]. It involves stimulating an animal's brain, most typically a site in the limbic system, at an intensity which at first has no discernible effect. The duration of the stimulation is short and the animal is typically stimulated once per day. If this schedule is maintained, afterdischarges (AD's) will develop at the site of stimulation. Over a period of days or weeks the animal will go through approximately 5 stages culminating in a full clonic or tonic-clonic convulsion. Pinel, Van Oot, and Mucha [15] have found that seizures induced by withdrawal from chronic alcohol administration were exacerbated if seizures previously had been kindled in the amygdala. The purpose of the present experiment was to examine the effect of ethanol on kindled seizure thresholds.

METHOD

Animals

The animals used were 16 male, Long-Evans rats weighing between 300–350 g at the time of surgery. The animals were housed in individual cages, in a vivarium with a 12 hr light–dark cycle (on 0600, off 1800). Food and water were provided ad lib, except as described below.

Apparatus

EEG recordings were made with a Grass model 7p511g EEG amplifier and 7dwu8p oscillograph. For brain stimulation a Grass model S 48 stimulator was used with a Grass model PSIU 6B photoelectric stimulus isolation unit with a constant current output. All EEG recordings were made in a 55 cm by 30 cm by 30 cm chamber shielded with brass hardware cloth.

Surgery

All animals were anesthetized with an intraperitoneal (IP) injection of a 50 mg/kg dose of sodium pentobarbital (Nembutal). The animals were placed in a stereotaxic instrument, a midline incision was made on the scalp and the skin and muscle were retracted. A bipolar electrode made of twisted platinum iridium wire insulated with Teflon was implanted unilaterally, on the left side in half the animals and right side in the other half, into either the amygdala or ventral hippocampus. With the skull tilted 5.0 mm and using bregma as the zero point, the following coordinates were used, according to the atlas of Pellegrino and Cushman [12]: amygdala – posterior, 0.8 mm, lateral, + or −4.5 mm, ventral, 8.0 mm; ventral hippocampus – posterior, −3.8 mm, lateral + or −5.0 mm, ventral, 7.5 mm. Four 0.80 stainless steel anchor screws were placed in the skull. Amphenol pins were crimped onto the end of the electrode and placed in a connector strip which was cemented, with dental acrylic, to the skull surface. The wound was sutured closed and the animals were returned to their home cage for a one week recovery period.

Procedure

One week after surgery the kindling procedure was initiated. The EEG was recorded from each animal for approximately 30 sec. Animals were then disconnected from the EEG machine and connected to the stimulator via a rotary switch. A 1 sec train of square wave pulses, 50 pps, 1.0 msec duration, 400 μA, was then delivered to the animal. The animal was then reconnected to the EEG machine to determine the presence of AD's. The animal's behavior was also rated by the experimenter on a scale of 1–5 similar to that used by Racine [19] with a 5 being the occurrence of clonic convulsions. Once a stage 5 seizure occurred, stage 5 convulsions were elicited on 4 successive days, once per day.

Following the elicitation of 5 stage 5 convulsions, threshold testing began according to the technique of Racine [19]. Animals were stimulated at 80 μA (all other stimulation parameters were the same as before). If a seizure occurred, the intensity of the next stimulation was halved; if no seizure occurred, the intensity was doubled. Animals were then stimulated at a current level half-way between the intensity that elicited a stage 5 seizure and the intensity that did not until the difference was no more than 20% of the seizure-eliciting intensity. Stimulation for threshold testing occurred no more frequently than once every other day.

Once seizure thresholds were established, thresholds were reassessed, using the same procedure, with the animal having been injected IP with saline, or a 600 or 1600 mg/kg dose of 25% ethanol (w/v). The animals were deprived of food at least 4 hr prior to injection. Twenty min after injection of the appropriate solution the threshold test was carried out. Animals were injected and tested only once per day, no more frequently than every other day. Once the threshold under a given solution was established, the threshold for the next solution was assessed. All animals were tested under all solutions, with the order of solutions being counterbalanced. Finally, thresholds were reassessed without any injection.

Histology

Upon completion of all testing animals were anesthetized with chloroform, and perfused intracardially, with 0.9% saline followed by 10% Formalin. The brains were removed and stored in 10% Formalin for at least two days. Frozen sections, 50 μ thick, through the electrode tract were mounted on slides and stained with thionin.

RESULTS

Histology

One animal each in the hippocampal and amygdalar groups lost their electrode prior to completion of the experiment and were not included in the data analysis. The histological results are presented in Fig. 1. For the animals with amygdalar implants all electrodes were either in the basolateral or lateral nuclei. All electrodes for the hippocampal group were found to be in the ventral hippocampus. One of the animals in the hippocampal group that was kindled to stage 5 did not continue to have seizures upon stimulation. The reason for this disruption could not be determined. Due to this disruption, thresholds could not be determined for this animal and it was dropped from the experiment.

The two stimulation groups differed in the number of stimulations to reach stage 5, with the median for amygdalar group being 8.5 and for the hippocampal group, 18.0. A Mann-Whitney U test performed on the data was significant ($U = 3.0$, $p<0.02$). During the first several days of stimulation, AD's were not elicited in every animal. To determine whether the group differences were attributable to this variable, the number of AD's to a stage 5 seizure was analyzed, too. The median number of AD's to stage 5 was also significantly lower for the amygdalar group (md. = 5.0) than the hippocampal group (md. = 11.0) with $U = 4.5$ ($p<0.05$).

To determine whether seizure thresholds differed between groups and whether thresholds were altered as a function of the treatments, a 2 × 2, site by time of threshold test (i.e. original vs. final threshold) analysis of variance was performed on the stage 5 seizure thresholds. The amygdalar group ($\bar{X} = 43.1$ μA) has a significantly lower threshold, $F = 26.1$, $p<0.001$, than the hippocampal group ($\bar{X} = 139.0$ μA). The original thresholds were not significantly different from the final thresholds, $F<1.0$. Finally, there was no site by time interaction, $F<1.0$.

As seen in Table 1 the thresholds for AD's and for stage 5 convulsions were not always the same. For the amygdala stimulated animals the saline and 600 mg/kg ethanol injections did not appear to yield any difference in AD and motor seizure threshold. The 1600 mg/kg ethanol injection yielded an increase in both the AD and motor seizure threshold with the greater increase occurring in the latter. For the hippocampus stimulated animals the mean thresholds for the 600 mg/kg ethanol injection appear to be slightly lower than the saline thresholds, while the 1600 mg/kg ethanol injection caused a marked increase in seizure thresholds. For most injections of the 1600 mg/kg dose the motor seizure thresholds appear to be higher than the AD seizure thresholds. Although stage 5 seizures always accompanied AD's for 11 of 13 animals for saline injections and for 9 of 13 animals for the 600 mg/kg injection, for the 1600 mg/kg injection only 2 of 13 animals had a stage 5 seizure every time an AD occurred.

Because the amygdalar group had significantly lower initial thresholds it is possible that any change in absolute thresholds for the two groups might be a function of the

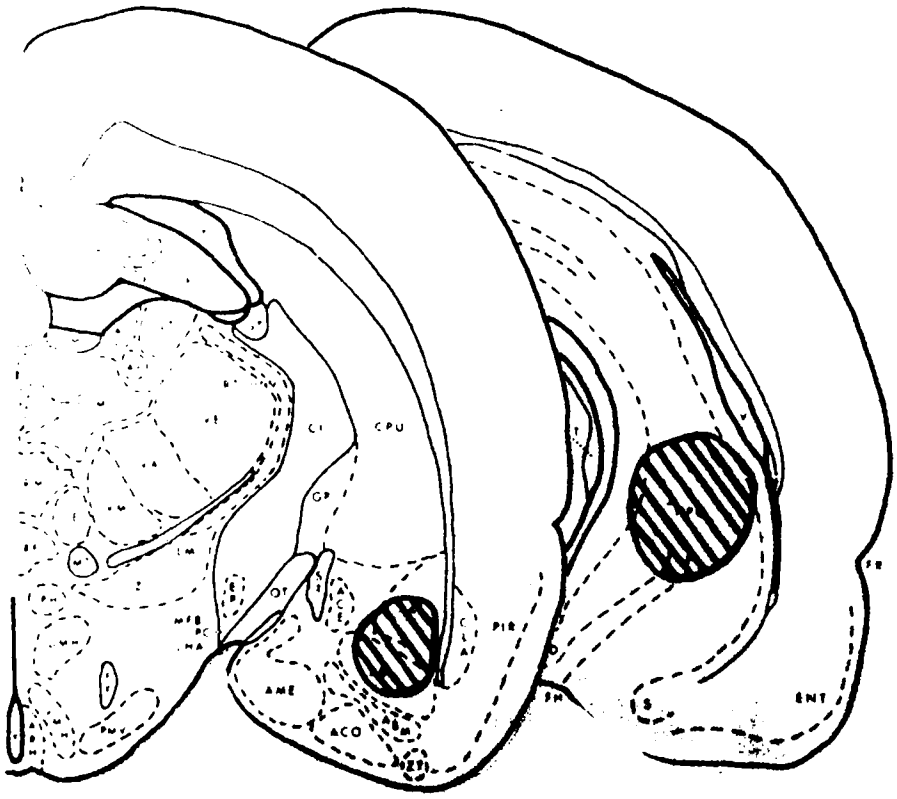

FIG. 1. Location of electrodes for amygdala (left) and hippocampal implants.

TABLE 1

AD AND MOTOR SEIZURE THRESHOLDS FOR THE AMYGDALA AND VENTRAL HIPPOCAMPUS

	Amygdala	Ventral Hippocampus
Saline		
AD	46.7	121.8
Motor	46.7	139.0
600 mg/kg		
AD	46.2	110.4
Motor	46.2	135.2
1600 mg/kg		
AD	57.9	183.6
Motor	67.2	302.8

TABLE 2

RATIOS OF SEIZURE THRESHOLDS FOR THE 600 AND 1600 MG/KG INJECTIONS RELATIVE TO THE THRESHOLDS FOR THE SALINE INJECTION

	600 mg/kg	1600 mg/kg
Amygdala		
AD	0.96	1.23
Motor	0.96	1.45
Ventral Hippocampus		
AD	1.03	1.61
Motor	0.99	2.01

initial threshold. To control for this, a ratio was computed for the 600 and 1600 mg/kg dose thresholds relative to the saline AD and motor seizure threshold (see Table 2). A 2 x 2 site by solution analysis of variance was then performed on these data. This yielded a significant effect for site, $F = 4.95$, $p<0.05$, for dose $F = 67.7$, $p<0.001$, and for the site by dose interaction, $F = 11.22$, $p<0.01$, for the AD thresholds. For motor seizure thresholds the F's were 8.2 ($p<0.025$), 50.1 ($p<0.001$), and 6.15 ($p<0.05$) for the site, dose, and site by dose interaction, respectively. Thus, the 1600 mg/kg dose raised the seizure threshold significantly more than the 600 mg/kg dose. Further, the thresholds for

the hippocampal group were raised significantly more by the 1600 mg/kg dose than were the thresholds for the amygdala group. Finally, AD durations at the AD and motor seizure thresholds were analyzed. No differences were observed for either threshold for any of the groups.

DISCUSSION

The effect of ethanol on seizure thresholds of the amygdala and hippocampus appear to parallel the effects seen when electroconvulsive shock is used to induce seizures (i.e. it increased seizure thresholds at higher doses). This elevation of both AD and motor seizure (i.e. stage 5) thresholds was found to be greater for the ventral hippocampus than for the amygdala. Further, the partial dissociation of AD's and motor seizures by ethanol occurred significantly more often in the ventral hippocampus.

The elevation of limbic system seizure thresholds by ethanol is in agreement with the study by Baker and Benedict [2]. However, in the study by Perrin, *et al.* [13] it was reported that the administration of ethanol had relatively little effect on hippocampal EEG while markedly suppressing activity in the cortex, hypothalamus, reticular formation, and amygdala. Further, Guerrero-Figueroa, *et al.* [9] observed that chronic ethanol administration had a relatively weaker suppressant effect on epileptogenic activity induced in the limbic system than in other areas. It should be kept in mind, however, that a markedly different technique for inducing seizures was employed in the present study (i.e. the measurement of seizure thresholds which were associated with the initiation of electrical stimulation). Although the techniques were markedly different, the present study does appear to parallel the observation by Perrin, *et al.* [13] that the ventral hippocampus is differentially affected by ethanol as compared to the amygdala. Whether this difference in kindled seizure thresholds occurs in extralimbic structures remains to be seen.

The observation of a dissociation between the occurrence of AD and motor seizures appears to be somewhat unique. It is typically reported that once seizures are kindled, AD's are usually associated with convulsive activity [7]. In studies on the anticonvulsant effect of THC on kindled seizures this dissociation was observed in several animals, but it was suggested that this observation was atypical [4,6]. Also, the results of the present study suggest that this dissociation is more likely to occur with seizures kindled in the ventral hippocampus as compared to the amygdala.

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