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Decreased Voluntary Ethanol Selection in Amygdala-Kindled Rats

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LINSEMAN, M. A., G. COTTRELL AND W. M. BURNHAM. *Decreased voluntary ethanol selection in amygdala-kindled rats.* PHARMACOL BIOCHEM BEHAV **48**(1) 31-36, 1994.—Kindled and control rats were exposed to either ethanol or dextrose solutions in the limited access paradigm, a paradigm that allows access to the test solution for only 1 h each day. Limited access trials were initiated either 24 h or 30 days after the fifth stage 5 seizure had been elicited in the kindled subjects. As previously reported, increased voluntary ethanol selection was observed in the limited access paradigm. Kindled subjects, however, ingested significantly less ethanol than controls. This difference was found both when limited access trials were started 24 h after the last seizure and when they were started 30 days after the last seizure. Kindled and control subjects did not differ in their intake of dextrose solutions.

Ethanol Kindling Voluntary alcohol selection Limited access paradigm

IN the kindling model, the repeated elicitation of focal discharge leads to the gradual development of generalized seizures with bilateral clonic convulsions (11,17,18,21,22). Generalized kindled seizures do not regress to their focal form even when stimulation is withheld for weeks or months (11, 17,18,21,22). It appears, therefore, that the kindling process is associated with brain changes which are long lasting or permanent in nature.

It has been suggested that the brain changes induced by kindling may affect interictal—as well as ictal—behavior. In cats, a large body of work by Adamec has demonstrated postkindling changes in aggressive and defense behaviors (1-4). In rats, postkindling changes have been reported in avoidance learning (8,16), locomotory behavior (10,12), predatory attack [(15); although see (7)], aggressiveness (19), and willingness to engage in punished behavior (28). These changes have typically been seen in subjects that have experienced 5-10 generalized seizures. In some cases, they have been observed in tests initiated a month or more after the last seizure (1,12).

The limited access paradigm is a procedure for producing long-term increases in voluntary alcohol selection in rats. It has been known for some time that rats increase their voluntary ethanol selection when ethanol is present on a discontinuous basis (5,6,25-27). In the limited access paradigm, rats are allowed access to gradually increasing concentrations of ethanol for 1 h a day, with pure water being offered as an alternate (13,14,24). Under these conditions, rats begin to choose ethanol over water, and voluntarily ingest significant amounts (13,14,24).

In 1975, Pinel and Mucha (20), working in an alternate-day discontinuous access paradigm, found that a series of electroconvulsive shock (ECS) seizures—administered just before or during discontinuous access trials—eliminated the increase in ethanol selection usually seen in this paradigm. These experiments further showed that the ECS effect was not due to conditioned taste aversion, and that similar results could be obtained when saccharin was substituted for ethanol (20).

The present study was designed to determine whether kindled seizures, like ECS seizures, would block the increased ethanol selection seen with discontinuous access presentations. Kindled and control rats were given a choice of water or ethanol solution in the 1-h limited access paradigm. Limited access trials were began either 24 h, or—because kindling-related effects may be permanent—30 days after the last kindled sei-

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TABLE 1
PROCEDURAL FLOW CHART

Subjects acclimate to vivarium
↓ (1 week minimum)
Implantation of electrodes/recovery
↓ (2 weeks)
Kindling or matched handling
↓ (approximately 1 month)
Interval of 24 h or 30 days
↓ (24 h/30 days)
Entry into limited access paradigm (6 weeks)

zure. Each test with ethanol was paralleled by a similar test in which dextrose (calorically matched) was substituted. It was hoped that this series of experiments would indicate: a) whether kindled seizures, like ECS seizures, block the discontinuous-access effect; b) whether this blockade, like kindling, is long lasting; and c) whether it is specific to ethanol.

METHOD

Overall Procedure

Table 1 presents a procedural flow chart for the whole experiment.

Subjects

One hundred and twenty male Wistar rats (275–300 g, Canadian Breeding Farms, Montreal, P.Q.) served as subjects. Subjects were housed individually in hanging wire cages in a vivarium with a 12-h reversed light : dark cycle (lights on at 1700 h; lights off at 0500 h). The reversed cycle was employed so that limited access testing could conveniently be done in the dark phase, when the animals are more active. Ad lib food (Purina Rat Chow) and water were available at all times. Each subject was randomly assigned to one of the following treatment groups:

1. Kindled with the last seizure 24 h before exposure to ethanol or dextrose in the limited access paradigm (kindled 24).
2. Matched handling with the last seizure (in kindled partners) 24 h before exposure to ethanol or dextrose in the limited access paradigm (control 24).
3. Kindled with the last seizure 30 days before exposure to ethanol or dextrose in the limited access paradigm (kindled 30).
4. Matched handling with the last seizure (in kindled partners) 30 days before exposure to ethanol or dextrose in the limited access paradigm (control 30). In groups that were destined for a 30-day wait after kindling, procedures were initiated 30 days earlier, so that all drinking trials began at the same time.

Surgery

In preparation for kindling, each subject was implanted with a single bipolar electrode (MS 303/1, Plastics One Co., Roanoke, VA) aimed at the basolateral amygdala. Implantations were done under pentobarbital anesthesia (50 mg/kg, IP), using standard stereotaxic techniques (23) and the following coordinates: posterior (bregma) −1.0 mm; lateral (right) −

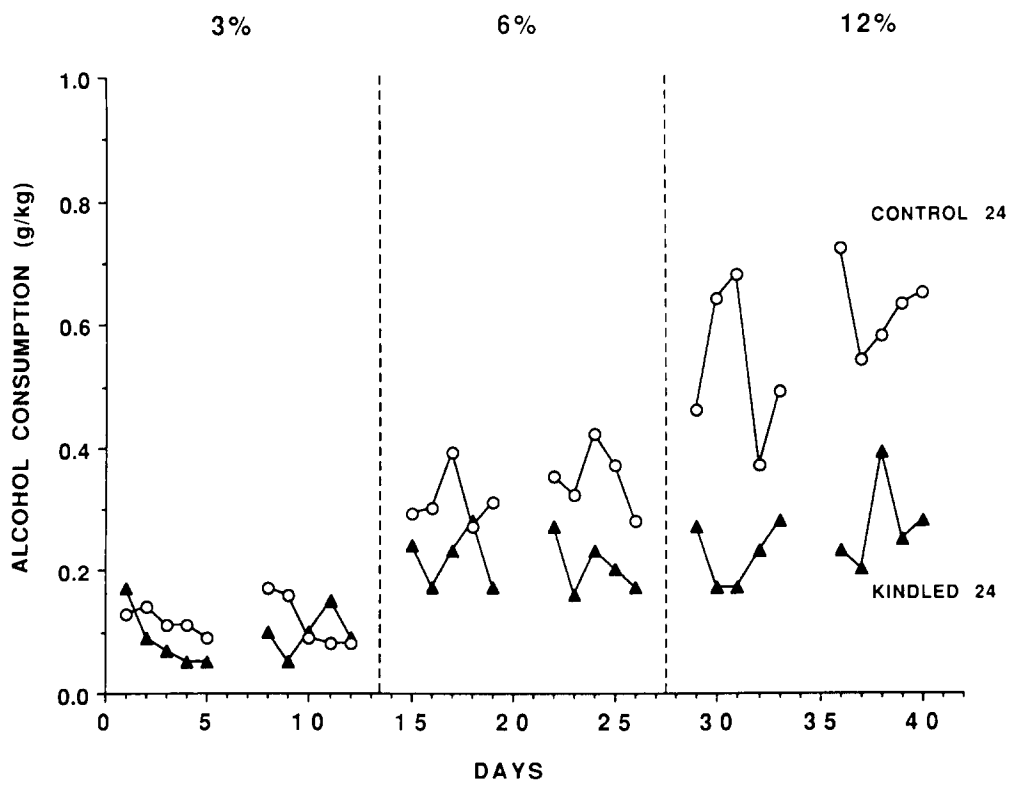

FIG. 1. Mean daily consumption of ethanol in subjects that received their last kindled seizure (or matched handling) 24 h before entry into the limited access paradigm.

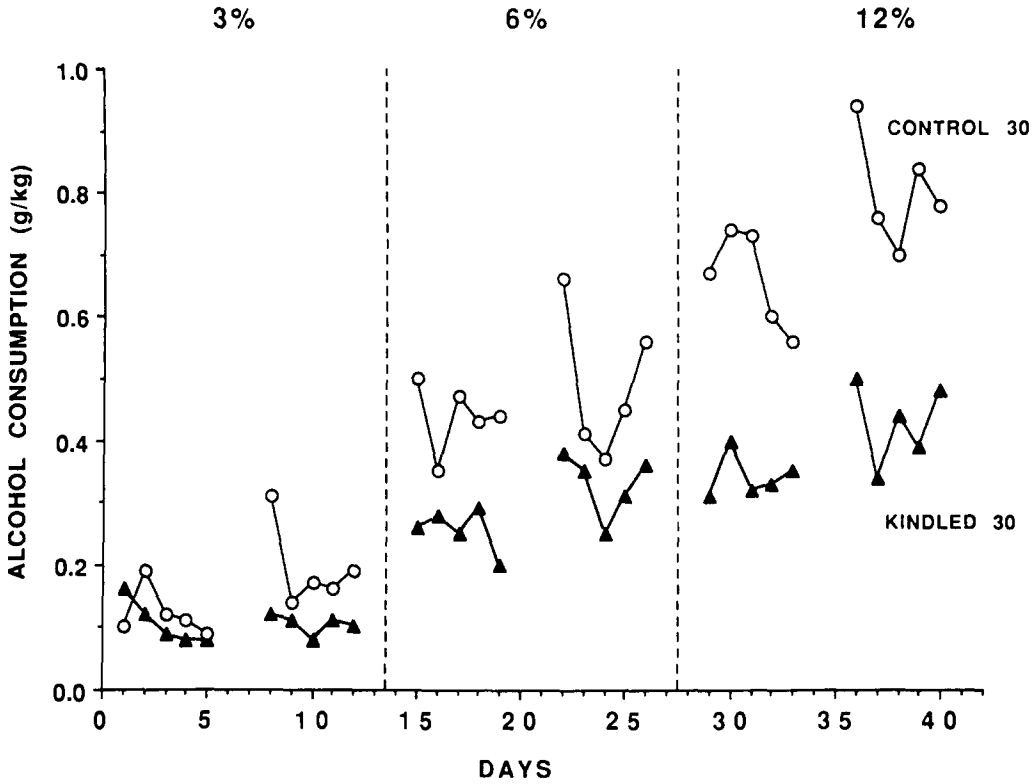

FIG. 2. Mean daily consumption of ethanol in subjects that received their last kindled seizure (or matched handling) 30 days before entry into the limited access paradigm.

4.8 mm; ventral (skull surface) −8.5 mm; incisor bar elevation above ear bars −5 mm.

Kindling Procedure

Two weeks following surgery, kindling or matched handling trials were begun. Subjects in the kindling groups received daily amygdaloid stimulation (5 days per week) until they had exhibited five consecutive rearing and falling or stage 5 (21) convulsions. An S88 stimulator (Grass Instrument Company, Quincy, MA) was used to provide a 1-s train of 1-ms square-wave pulse pairs (positive and negative going) at a frequency of 60 Hz and an intensity of 400 μA (peak to peak). Subjects in the control groups received identical handling, but no stimulation.

Limited Access Paradigm

Either 24 h or 30 days after the kindled subjects had exhibited their 5th stage 5 seizures, trials in the limited access paradigm were initiated. Trials were administered once per day (5 days a week) for 6 weeks. Subjects were removed from their home cages, weighed, and then placed in individual drinking cages located on a separate rack in the colony room. No food was available, but modified Richter tubes offered a choice of water or ethanol solution. (In dextrose studies, the choice was water or dextrose solution.) Following the 1-h drinking session, subjects were returned to their home cages, and the amounts of fluid they had consumed were measured. Half of the subjects (randomized across groups) commenced their drinking trials at 0800, while the other half (randomized across groups) commenced their trials at 1200. Because trials took place in the dark half of the reversed light : dark cycle, a small red light was used to illuminate the rooms during data collection.

During the first 2 weeks of limited access trials, the concentration of ethanol was 3% w/v in tap water. During the next 2 weeks, the ethanol concentration was increased to 6%, and during the final 2 weeks, the ethanol concentration was increased to 12%. The concentrations of dextrose used during these periods were 5.25%, 10.5%, and 21%, respectively (caloric equivalents of 3%, 6%, and 12% ethanol). Solutions were mixed fresh daily, and the positions of the ethanol/dextrose and water tubes were alternated to control for possible position preferences.

Histology

At the conclusion of testing, every tenth subject was sacrificed by an overdose of pentobarbital, and perfused through the heart with physiological saline followed by 10% formalin. The brains were frozen, sliced in 30 μM sections, and stained with thionin. Histological examination revealed that all electrode tips had been located in or near the basolateral amygdala.

RESULTS

Figures 1 and 2 show mean daily ethanol consumption (g/kg) for kindled and control subjects. Data for the 24-h groups are shown in Fig. 1 and data for the 30-day groups are shown in Fig. 2. As indicated, control subjects at both time periods

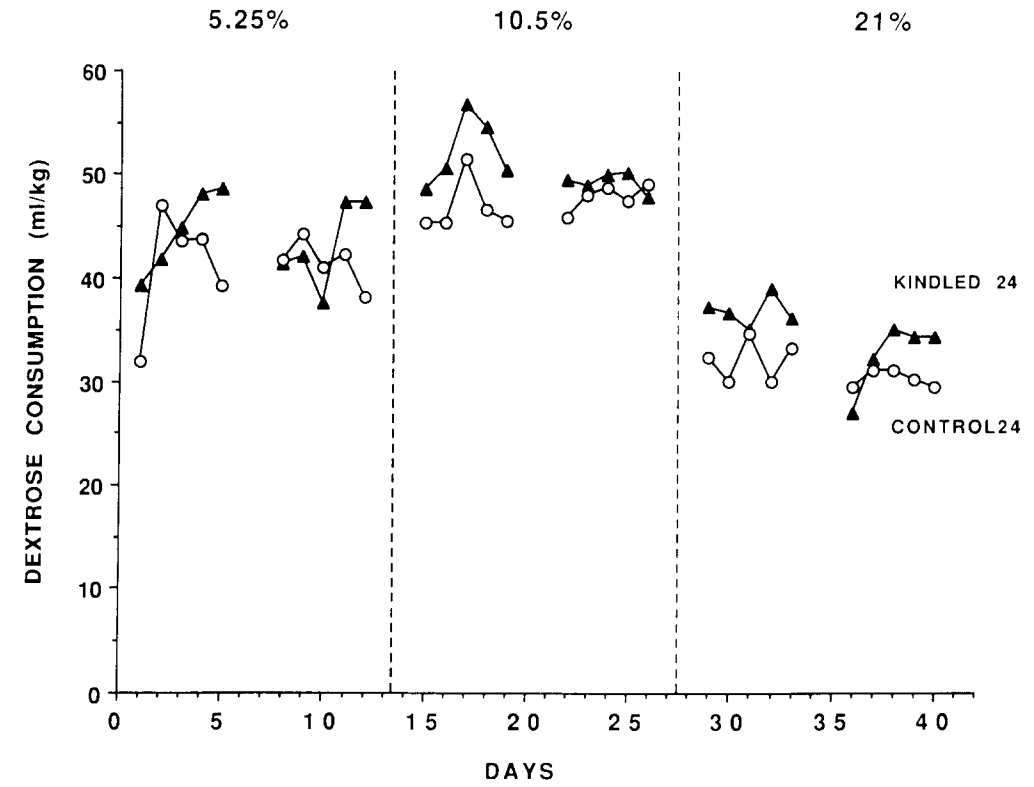

FIG. 3. Mean daily consumption of dextrose in subjects that received their last kindled seizure (or matched handling) 24 h before entry into the limited access paradigm.

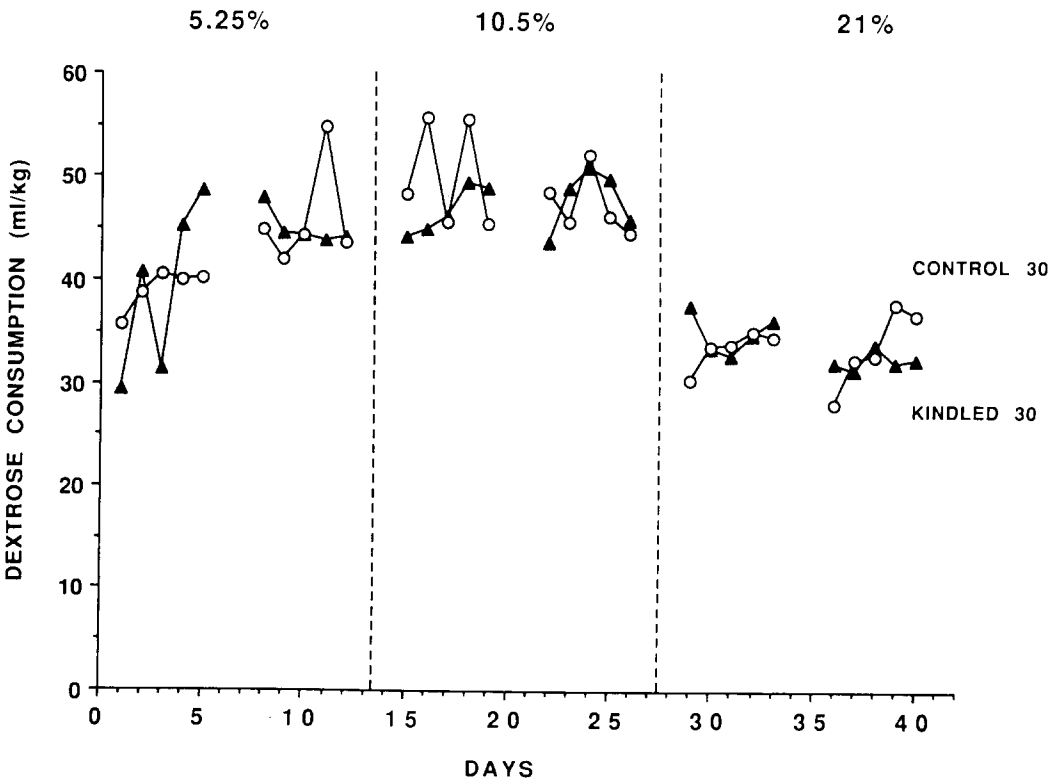

FIG. 4. Mean daily consumption of dextrose in subjects that received their last kindled seizure (or matched handling) 30 days before entry into the limited access paradigm.

increased their selection of ethanol. By the end of 6 weeks, consumption in the controls averaged 0.7–0.8 g/kg/day. Kindled subjects also increased their consumption, but to a lesser extent. At the end of 6 weeks, kindled subjects were drinking approximately 0.3–0.5 g/kg/day. An analysis of variance revealed a significant effect of kindling, $F(1, 52) = 8.562$, $p < 0.005$, but no significant effect of time of entrance into the paradigm, $F(1, 52) = 0.067$, $p > 0.05$. Thus, kindled subjects showed less voluntary ethanol selection, and this effect was similar whether the trials started 24 h or 30 days after the last seizure. There was also a significant interaction between kindling and concentration, $F(2, 52) = 8.892$, $p < 0.0005$, indicating that the largest differences between kindled and control subjects occurred in the later trials when ethanol concentrations were higher.

Figures 3 and 4 show mean daily dextrose consumption (g/kg) for kindled and control subjects. Data for the 24-h groups are shown in Fig. 3, and data for the 30-day groups are shown in Fig. 4. As indicated, there was a high level of dextrose selection that began in the early trials and continued throughout the series, although it was somewhat lower at the highest concentration. Consumption was similar in all groups. An analysis of variance revealed no significant difference between kindled and control subjects, $F(1, 52) = 0.378$, $p > 0.05$, and no effect of time of entry into the paradigm, $F(1, 52) = 0.74$, $p > 0.05$. There was no significant interaction between kindling and concentration, $F(2, 104) = 2.987$, $p > 0.05$. Thus, although kindling affected the level of voluntary ethanol selection, it did not affect the level of voluntary dextrose selection.

DISCUSSION

The present study was designed to determine whether a series of kindled seizures would block the increased ethanol selection usually observed in the limited access paradigm. It was found that kindled subjects displayed significantly lower levels of ethanol selection than matched control animals. Thus, kindled seizures do appear to decrease the effects of limited access.

These data complement and extend a previous report by Pinel and Mucha (20), who found that ECS seizures blocked the increase in ethanol selection usually seen in a 24-h discontinuous access paradigm. Although the two studies differed widely in their procedures (see below), both found an inhibitory effect of convulsions on discontinuous access ethanol selection. This effect appears to be a robust one, which might be seen after repeated convulsions of other types as well.

A second goal of the present study was to determine whether the effect of seizures on ethanol selection was long lasting—like kindling. We, therefore, tested a group of subjects in which 30 days had elapsed between the last kindled seizure and the first limited access trial. By 30 days, it is believed that most of the transitory effects of seizures have dissipated (9). A significant depression of voluntary ethanol selection was still observed even in subjects that had waited 30 days before entering the limited access paradigm. Thus, it appears that the effect of kindled seizures on alcohol selection is long lasting and possibly permanent. These data are also in agreement with the previous report of Pinel and Mucha (20), who found that the enhancement of ethanol selection by alternate-day access was suppressed for 20–30 days after discontinuation of ECS seizures. Together, these two studies suggest that convulsive seizures may have a very long-lasting effect on ethanol selection.

A final goal of the present experiment was to determine whether the effect of kindled seizures was specific to ethanol selection. Pinel and Mucha found post-ECS decreases in both ethanol and saccharin consumption in the 24-h discontinuous access paradigm. Instead of saccharin, we used dextrose—a caloric match for ethanol—and found no kindled/control differences at either 24 h or 30 days. This difference between our findings and Pinel's may relate to the different types of seizures involved, or to the differences between saccharin and dextrose. It may also relate to a number of procedural differences between the two studies: Pinel used 24-h access, whereas we used 1-h; Pinel used 20% ethanol, whereas we graded up from 3%; Pinel interspersed seizure trials and drinking trials—and so forth. The specificity question might be addressed in a future series of studies involving standardized tests in the limited access paradigm and a variety of different test substances, including both saccharin and dextrose.

Future tests might also address a question which the present data cannot resolve. In our procedure, the higher ethanol doses coincide with the later test trials, and the increased ethanol selection observed in these trials might result either from limited access exposure, from increased ethanol concentration, or from a combination of the two. Future tests might involve kindled and control subjects exposed to a constant concentration of ethanol.

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