ELECTRICAL AMYGDALA KINDLING IN ALCOHOL-WITHDRAWAL KINDLED RATS

JAKOB ULRICHSEN*, DAVID P. D. WOLDBYE, TORSTEN M. MADSEN, LARS CLEMMESEN, STEVEN HAUGBØL, CHRISTIAN H. OLSEN, HENNING LAURSEN1, TOM G. BOLWIG and RALF HEMMINGSEN2

Neuropsychiatric Research Group, Department of Psychiatry 0-6234, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Institute of Neuropathology, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen and Department of Psychiatry, Bispebjerg Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen NV, Denmark

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Abstract — Repeated alcohol withdrawal has been shown to kindle seizure activity. The purpose of the present investigation was to study electrical amygdala kindling in rats previously exposed to alcohol-withdrawal kindling. In three independent experiments, male Wistar rats were subjected to multiple episodes each consisting of 2 days of severe alcohol intoxication and 5 days of alcohol withdrawal. In the first experiment, the alcohol-withdrawal kindled animals were divided into two groups depending on whether spontaneous alcohol-withdrawal seizures were observed in episodes 10–13. In the second and third experiments, the alcohol-withdrawal kindled animals were compared to a group in which alcohol-withdrawal kindling was prevented by diazepam treatment during the withdrawal reactions in order to discriminate between the effect of withdrawal and intoxication. Electrical kindling was initiated 28–35 days after the last alcohol dose by exposing the animals to daily electrical stimulations of the right amygdala. The results showed that amygdala kindling was facilitated in alcohol-withdrawal kindled animals which showed spontaneous withdrawal seizure activity, compared with animals exposed to multiple episodes of alcohol withdrawal which did not develop withdrawal seizures or with animals exposed to a single episode of alcohol intoxication. When compared to the control group, the alcohol-withdrawal kindled group with seizures also kindled at a faster rate, but the difference did not reach statistical significance and therefore the results must be regarded as preliminary at present.

INTRODUCTION

Kindling is a process in which repeated applications of initially subconvulsive stimulations eventually produce motor seizures and electroencephalographic (EEG) alterations of progressive severity. The phenomenon, which is long-lasting, if not enduring, was originally described by Goddard et al. (1969), who repeatedly applied low intensity electrical currents to local brain regions of rats, cats, and monkeys (electrical kindling). Since then, numerous studies have confirmed the findings of Goddard et al. (1969) and have demonstrated that other species, such as frogs, reptiles, mice, and rabbits can also be kindled (reviewed by Racine, 1978). It has also been shown that seizure activity can be kindled pharmacologically by repeated administrations of different drugs such as cocaine, lidocaine, glutamate, and the inverse benzodiazepine agonist FG 7142 (Little et al., 1987; Post et al., 1988; Stephens and Weidmann, 1989; Weiss et al., 1989; Croucher and Bradford, 1990).

Recently, we demonstrated that repetition of alcohol withdrawal in rats was accompanied by an increased seizure activity during later withdrawal reactions; a process which could be prevented by blocking the early withdrawal reactions with phenobarbital or diazepam (Ulrichsen et al., 1992, 1995). These findings are in agreement with animal studies showing increased convulsive withdrawal behaviour during repeated episodes of alcohol intoxication and withdrawal (Clemmesen and Hemmingsen, 1984; Becker and Hale, 1993; Kokka et al., 1993; Becker, 1994) and with clinical studies which demonstrated a positive relationship between the number of previous withdrawal reactions and the risk of developing withdrawal...
seizures during oncoming withdrawal reactions (Brown et al., 1988; Lechtenberg and Worner, 1992; Booth and Blow, 1993; Moak and Anton, 1996). Altogether, these studies strongly support the hypothesis put forward by Ballenger and Post (1978) that convulsive and psychotic withdrawal behaviour (i.e. delirium tremens) can be kindled by repeated alcohol withdrawal.

A unique feature of electrical kindling is that kindling in one brain region facilitates kindling in a secondary focus. This transfer effect can be demonstrated even if the primary kindling site is destroyed by chemical or surgical procedures (Goddard et al., 1969; Racine, 1978). A similar transfer effect between electrical kindling and chemical kindling has been demonstrated by Croucher and Bradford (1989), who showed that chemically kindled animals required significantly fewer electrical stimuli to re-kindles than control animals.

In order to understand the basic mechanisms of alcohol-withdrawal kindling, it is important to study whether transfer mechanisms (or cross-sensitivity) exist between electrical kindling and alcohol-withdrawal kindling, but unfortunately the literature concerning this matter is limited. Carrington et al. (1984) exposed previously alcohol-withdrawal kindled rats to electrical kindling of the amygdala. These authors did not find significant differences in the electrical kindling rate between alcohol-withdrawal kindled animals and controls. In another study, alcohol-withdrawal kindling facilitated electrical kindling in the inferior colliculus, whereas electrical kindling in the amygdala was retarded (McCown and Breese, 1990). Whether alcohol-withdrawal kindling is accelerated following electrical kindling has never been investigated, but Pinel et al. (1975) demonstrated an intensified alcohol-withdrawal reaction (single episode) in amygdala-kindled animals.

The purpose of the present experiments was to study how electrical kindling of the amygdala is affected by previous alcohol-withdrawal kindling. The hypotheses to be tested were that amygdala kindling would be facilitated in alcohol-withdrawal kindled animals, whereas animals exposed to multiple episodes of alcohol intoxication in which the withdrawal reactions were blocked by diazepam treatment would kindle at the same rate as control animals, as diazepam has been shown to prevent alcohol-kindling (Ulrichsen et al., 1995).

MATERIALS AND METHODS

Alcohol-withdrawal kindling

Male Wistar rats (Møllegaard, Køge, Denmark) weighing 300 g (experiment 1) and 155–200 g (experiments 2 and 3) were housed in a room with a 12 h light/12 h dark cycle (lights on at 07:00) and had free access to food pellets and water. During each intoxication period (see below), alcohol was administered five times a day between 08:00 and 24:00 by intragastric intubation (Majchrowicz, 1975). Before each alcohol administration session, the degree of alcohol intoxication was assessed by using the following rating scale:

0. Neutral: no signs of intoxication.
1. Sedation: reduced muscle tone, dulled appearance and slow locomotor activity, but no impairment of gait or coordination.
2. Ataxia 1: slight gait impairment and slight motor incoordination, but able to elevate abdomen and pelvis.
3. Ataxia 2: clearly impaired staggering gait and impaired motor coordination, some elevation of abdomen and pelvis.
5. Loss of righting reflex (LRR): unable to right itself when placed on its back, other reflexes still present.
6. Coma: no signs of movement; no response to pain stimuli; no blinking reflex; spontaneous breathing.

The alcohol dose was adjusted individually to the degree of intoxication. Neutral rats received 5–7 g/kg, whereas animals with LRR received 0–1 g/kg. The alcohol solution consisted of: ethanol 200 g/l, sucrose 300 g/l; and multivitamin mixture 4 ml/l in Ringer's solution, the sucrose being added in order to prevent hypoglycaemia and ketosis (Hemmingsen and Chapman, 1980). Control animals were matched with the alcohol-withdrawal kindled animals and received an isocaloric amount of sucrose instead of alcohol and the same amount of water and food as the alcohol-withdrawal kindled animals. All animals which survived the multiple intoxication cycles (see later) and the isocalorically fed control animals were in good health as assessed by general appearance, behaviour, body weight and consumption of food and water.

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The non-convulsive withdrawal behaviour was assessed as previously described (Ulrichsen et al., 1986). Briefly, the three individual items intentional tremor, rigidity, and hyperactivity/irritability were each scored on a four-level scale (0–3) and the sum of these scores (0–9) was used as a quantitative measure of the severity of the non-convulsive withdrawal reaction.

**Design**

Alcohol-withdrawal kindling was performed by subjecting the animals to multiple episodes each consisting of 2 days of severe alcohol intoxication followed by 5 days of alcohol withdrawal (Clemmesen and Hemmingsen, 1984). The effect of previous alcohol-withdrawal kindling on subsequent electrical amygdala kindling was investigated in three separate experiments:

**Experiment 1.** Forty animals were exposed to 13–16 episodes of repeated alcohol intoxication and alcohol withdrawal. During episodes 10–16, all animals were recorded on video-tape for 120 min 10–14 h into the withdrawal reaction in order to register spontaneous seizure activity. Seizures observed outside recording periods, i.e. in the cages, were additionally registered. Spontaneous seizures (one or more) were observed in six out of the 25 surviving animals. One of these seizure animals died of alcohol intoxication during episode 13. After each of episodes 13–16, we selected two to five animals for electrode implantation. Animals in which we had observed one or more seizures were automatically selected for operation and comprised the alcohol-withdrawal kindled group with seizures. Among the remaining animals in which seizures were not observed (the alcohol-withdrawal kindled group without seizures), a total of 10 animals were randomly selected for operation after episodes 13–16. The assignment of these animals for operation was performed such that the average number of previous sucrose episodes was similar to the number of alcohol intoxication episodes in the alcohol-withdrawal kindled groups. Fifteen to 20 days post-operation, the single episode animals were operated after sucrose administration in episodes 13–16. The assignment of animals from the latter group for operation was performed such that the average number of previous sucrose episodes was similar to the number of alcohol intoxication episodes in the alcohol-withdrawal kindled groups. Fifteen to 20 days post-operation, the single episode group was exposed to a first time episode of 2 days of severe alcohol intoxication. In this group, electrical kindling was performed 28–35 days after the last alcohol dose, whereas the controls were electrically kindled concomitantly with the alcohol-withdrawal kindled animals selected for operation after episode 16.

**Experiment 2.** Two experimental groups were included. The first, the alcohol-withdrawal kindling group (n = 40), was subjected to eight episodes of repeated alcohol intoxication and alcohol withdrawal. The other group (the multiple intoxication group, n = 40) was exposed to the same alcohol regimen, but following each alcohol intoxication episode, the withdrawal reaction was blocked by diazepam treatment (see later) in order to prevent alcohol-withdrawal kindling in this group (Ulrichsen et al., 1995). Following the
repeated alcohol administration, we randomly selected 17, 10, and seven animals from the control group, the alcohol-withdrawal kindled group, and the diazepam group, respectively, for amygdala kindling. Electrodes were implanted 19–30 days post-alcohol such that the average time from the last alcohol dose to the operation was the same across the three groups. Amygdala kindling was initiated after a post-operative recovery period of 3–14 days (i.e. exactly 33 days after last administration of alcohol in all rats).

Experiment 3. This was performed because the results of the second experiment were inconclusive due to exclusion of several animals. Thus the design was identical to the design in the second experiment, except that in addition to the alcohol-withdrawal kindling group \((n = 45)\) and the multiple intoxication group \((n = 45)\) another group was included in order to control for the effects of the diazepam treatment per se. This group (the diazepam group; \(n = 25\)) was fed isocalorically with the alcohol-withdrawal kindled group and received the same amount of diazepam as the multiple intoxication group. Eight animals from each group were randomly selected for electrode implantation. Blood-alcohol concentration was measured as described above on the second day of alcohol intoxication episode 8 in six randomly selected animals from the total number of animals in each of the two alcohol-treated groups.

Diazepam treatment

Diazepam was administered during each of the eight alcohol-withdrawal episodes as previously described (Ulrichsen et al., 1995). Briefly, diazepam \((5 \text{ mg/ml})\) was administered i.p. at 8, 11, and 15 h after the last alcohol dose. The clinical condition of the animals was assessed before each diazepam administration and intoxication was rated using the intoxication scale described above (Majchrowicz, 1975). At any time, intoxicated animals which were rated ataxia 3 or more, ataxia 2, ataxia 1 or sedation received 0 mg/kg, 0–15 mg/kg, 15–25 mg/kg, and 25 mg/kg diazepam i.p., respectively. Animals which showed any signs of alcohol withdrawal received 30 mg/kg diazepam, i.p. The animals typically received a total of 60–65 mg/kg diazepam per episode. In order to verify that the withdrawal reactions were in fact blocked by the diazepam treatment, the withdrawal reaction was assessed in a selected episode (episode 3) in experiment 2, 2 h after the second diazepam administration. The rating was done blindly on 17–19 randomly selected animals from both the two experimental groups and the alcohol-naive control animals.

Electrical kindling

The rats were placed in a stereotaxic apparatus under equithesin anaesthesia \((3.3 \text{ ml/kg, i.p.})\) and concentric, bipolar, stainless-steel electrodes \((0.25 \text{ mm}; \text{ Rhodes Medical Instruments, CA, USA})\) were chronically implanted into the right amygdala \((2.8 \text{ mm caudal to bregma, 5.0 mm lateral to the midline, 7.0 mm ventral to dura})\). The incisor bar of the stereotaxic apparatus was adjusted to position bregma and lambda in the same horizontal plane. The electrodes were secured to the skull with seven jeweller’s screws, covered with dental cement and positioned into a headplug assembly (Barry et al., 1989). Kindling was performed by daily application of electrical stimulations consisting of 2 s trains of 60 Hz, 1 ms, biphasic rectangular pulse waves. In the first experiment, the current was 200 \(\mu\text{A}\) in all the animals. In the second and third experiments the after-discharge threshold was determined on the first kindling day by increasing the current in 50 \(\mu\text{A}\) steps (starting at 50 \(\mu\text{A}\)) every 5 min until an after-discharge was evoked and this current was used during the remaining kindling sessions. Seizures were rated according to the severity scale of Racine (1972). Kindling was considered to be completed when an animal had experienced one (experiment 1) or a total of three (experiments 2 and 3) grade 5 seizures (tonic–clonic seizure with rearing and loss of balance).

Following completion of kindling, the rats were decapitated under halothane anaesthesia, their brains were rapidly removed, placed on dry ice, and stored at \(-80^\circ\text{C}\). Subsequently, coronal cryostat sections \((40 \mu\text{m})\) were cut through the area of the electrode tip, the sections were mounted on pregelatinized glass slides, dipped into a solution of Neutral Red \((1\% \text{ w/v})\) for 15 s, and coverslipped in Pertex. The position of the stimulating electrode was determined under microscope and only rats with electrode tips within the amygdala were included in the study.
Statistical analysis

Group effects on the number of kindling stimuli were analysed by one-way analysis of variance (ANOVA) while a posteriori group comparisons were performed using Duncan’s test. Data in which the requirements for using parametric statistics were not fulfilled were analysed by non-parametric methods. Thus, group effects on the after-discharge threshold and the alcohol-withdrawal score were analysed using the Kruskal–Wallis test, whereas multiple group comparisons were performed using the Mann–Whitney U-test. Data are presented as means ± SEM, except for the variables after-discharge threshold and alcohol-withdrawal score in which we report the median (and range). The level of significance was set to 5%. The statistical analyses were carried out by using SPSS 6.1 for Windows.

Drugs

Diazepam (Stesolid emulsion) was commercially obtained from Dumex-Alpharma, Denmark, whereas the anaesthetic equithesin and the multi-vitamin mixture were produced by the pharmacy at the Rigshospitalet, Denmark. Equithesin consisted of: pentobarbital, 9.7 mg/ml; ethanol, 76 mg/ml; chloral hydrate, 42.5 mg/ml; propylene glycole, 428 mg/ml; magnesium sulphate, 21 mg/ml in sterile water. The multivitamin solution consisted of: vitamin A, 4000 IE/ml; thiamine hydrochloride, 2 mg/ml; riboflavine sodium phosphate, 2.2 mg/ml; sodium hydrogen carbonate, 55 mg/ml; nicotinamide, 12 mg/ml; folic acid, 100 µg/ml; pyridoxine hydrochloride, 0.4 mg/ml; ascorbic acid, 100 mg/ml; ergocalciferol, 1600 IE; α-tocopherol acetate, 8 mg/ml; dexpanthenol, 2 mg/ml in distilled water.

Ethics

The study was approved by the Danish Animal Experiment Inspectorate, Ministry of Justice.

RESULTS

Experiment 1

During the alcohol-withdrawal kindling regimen, the number of animals was reduced from 40 to 25, due to overdosage of alcohol. During the final episode of alcohol dependence, the alcohol dose and the mean intoxication score were 23.9 ± 0.9 g/kg and 2.8 ± 0.1, respectively, in the single-episode group, 22.3 ± 0.9 g/kg and 3.0 ± 0.1, respectively, in the alcohol-withdrawal kindled group without seizures and 24.7 ± 2.2 g/kg and 2.7 ± 0.2, respectively, in the alcohol-withdrawal kindled group with seizures. The blood-alcohol concentrations were 3.9 ± 0.2, 4.1 ± 0.1, and 4.2 ± 0.1, respectively, whereas the corresponding intoxication levels were 4.3 ± 0.2, 4.0 ± 0.0, and 4.0 ± 0.3, respectively. The median (and range) of the maximal sum score of the alcohol-withdrawal reaction following the final episode of alcohol intoxication was 3.5 (2–5), 3 (3–5), and 4 (3–5), in the single episode group, the alcohol-withdrawal kindled group without seizures and the alcohol-withdrawal kindled group with seizures respectively. No significant differences were found between these withdrawal scores (Kruskal–Wallis test, P = 0.32).

In post-mortem examinations, we discovered three animals from the single episode group and one animal from the alcohol-withdrawal kindled group without seizures in which the electrodes were positioned outside the amygdala. These animals were excluded from the study. Consequently, the control group, the single episode group, the alcohol-withdrawal kindled group without seizures and the alcohol-withdrawal kindled group with seizures consisted of five, 12, nine, and five subjects, respectively. At the time of electrode implantation, the animals in these groups weighed 436 ± 15, 423 ± 9, 401 ± 22, and 439 ± 23 g, respectively.

The number of stimuli necessary to kindle the animals were significantly different across the four groups (Table 1). Multiple group comparisons revealed that this value was significantly decreased in the alcohol-withdrawal kindled group with seizures, compared with the single episode group and the alcohol-withdrawal kindled group without seizures (Duncan’s test). There was no significant difference in the number of kindling stimuli between the control group and the three experimental groups or between the alcohol-withdrawal kindled group without seizures and the single episode group. However, there was a trend towards a reduction of this variable in the alcohol-withdrawal kindled group with seizures, compared with the control group.

Experiment 2

During the eight episodes of alcohol depen-
Table 1. Electrical amygdala kindling of rats exposed to 14–17 episodes each consisting of 2 days of alcohol intoxication and 5 days of alcohol withdrawal (experiment 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of stimulations</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td></td>
<td>8.8 ± 1.8</td>
</tr>
<tr>
<td>Single episode (n = 12)</td>
<td></td>
<td>10.8 ± 1.2*</td>
</tr>
<tr>
<td>Alcohol-withdrawal kindling without seizures (n = 9)</td>
<td></td>
<td>9.2 ± 1.2*</td>
</tr>
<tr>
<td>Alcohol-withdrawal kindling with seizures (n = 5)</td>
<td></td>
<td>4.0 ± 1.3</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>F = 3.81; df = 3,27; P = 0.021</td>
</tr>
</tbody>
</table>

Alcohol-withdrawal kindled rats and rats exposed to a single episode of alcohol intoxication were exposed to daily electrical stimulations of the amygdala 28–35 days post-alcohol until one grade 5 seizure was observed. Values that differ significantly from the number of stimuli in the alcohol-withdrawal kindled group with seizures are indicated by *P < 0.05 (Duncan's test).

During the eight episodes of alcohol dependence, the numbers of alcohol-withdrawal kindled animals and multiple intoxication animals were reduced from 40 initially to 22 and 15, respectively. During the final intoxication episode (i.e. episode 8), the alcohol dose and the mean intoxication score were 26.0 ± 1.0 g/kg and 3.3 ± 0.07 respectively in the six alcohol-withdrawal kindled animals, and 25.8 ± 0.2 g/kg and 3.5 ± 0.1 respectively, in the three animals of the multiple intoxication group.

The mean (and range) of the sum score of the alcohol-withdrawal reaction in episode 3 in the alcohol-withdrawal kindled group, the multiple intoxication group and the control group, was 2 (0–4), 0 (0–0), and 0 (0–2), respectively (n = 17–19 per group). The Kruskal–Wallis test revealed significant group differences (P < 0.001). Multiple group comparisons showed that the withdrawal sum score in the alcohol-withdrawal kindled group was significantly higher than in both the control group and the multiple intoxication group (P < 0.001), whereas there was no significant difference in the sum score between the control group and the multiple intoxication group.

In post-mortem examinations, we discovered four animals from each group which had electrodes outside the amygdala. These animals were excluded from the study. Consequently, the control group, the alcohol-withdrawal kindling group, and the multiple intoxication group consisted of 13, six, and three subjects, respectively.

At the time of electrode implantation, the animals in these groups weighed 339 ± 3, 355 ± 9, and 333 ± 2 g respectively.

ANOVA indicated that the number of amygdala stimulations necessary to kindle the animals differed across the three groups, although this difference was not significant (P = 0.052) (Table 2). Post-hoc comparisons (Duncan’s test) revealed that the number of kindling stimuli was significantly decreased in the alcohol-withdrawal kindled group, compared with the control group. No significant differences in this parameter were found between the multiple intoxication group and the other two groups, but we detected a trend towards a decreased number of kindling stimuli in the alcohol-withdrawal kindled group compared with the multiple intoxication group. The median (and range) of the after-discharge threshold was 150 (100–350), 100 (100–500), and 100 (100–100) µA in the control group, the alcohol-withdrawal kindled group and the multiple intoxication group respectively. These values did not differ significantly across groups (Kruskal–Wallis; P = 0.24).

Experiment 3

During the eight episodes of alcohol dependence, the numbers of alcohol-withdrawal kindled animals and multiple intoxication animals were reduced from initially 45 to 31 and 35, respec-
Table 2. Electrical amygdala kindling of rats exposed to eight episodes each consisting of 2 days of alcohol intoxication and 5 days of alcohol withdrawal

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment 2 data</th>
<th>Experiment 3 data</th>
<th>Experiments 2 and 3 pooled data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of stimulations</td>
<td>No. of stimulations</td>
<td>No. of stimulations</td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
<td>19.7 ± 1.1</td>
<td>7</td>
</tr>
<tr>
<td>Alcohol-withdrawal kindling</td>
<td>6</td>
<td>13.3 ± 2.9*</td>
<td>7</td>
</tr>
<tr>
<td>Multiple intoxication</td>
<td>3</td>
<td>21.0 ± 3.6</td>
<td>6</td>
</tr>
<tr>
<td>Diazepam</td>
<td>6</td>
<td>23.5 ± 5.4</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA: $F = 3.46; df = 2,19; P = 0.053$

$F = 0.14; df = 3,22; P = 0.936$

$F = 0.22; df = 2,39; P = 0.800$

Two almost identical experiments were performed. In experiment 2, alcohol-withdrawal kindled rats and rats which were treated with diazepam during each withdrawal episode (the multiple intoxication group) were exposed to daily electrical stimulations of the amygdala 33 days post-alcohol until three grade 5 seizures were observed. In experiment 3, a group which received no alcohol but the same amount of diazepam as the multiple intoxication group was included. The mean ± SEM number of electrical stimulations for each experiment are presented as well as the pooled data from the control group and the two alcohol-treated groups. A statistically significant difference between the control group and the alcohol-withdrawal kindled group is indicated by $^*P < 0.05$ (Duncan’s test).

During the final intoxication episode (i.e. episode 8), the alcohol dose and the mean intoxication score were 24.4 ± 0.6 g/kg and 2.9 ± 0.05 respectively in the six alcohol-withdrawal kindled animals, and 25.6 ± 0.6 g/kg and 3.0 ± 0.06 respectively in the six animals of the multiple intoxication group. The blood-alcohol concentrations were 4.5 ± 0.1 and 4.8 ± 0.3 g/l in the alcohol-withdrawal kindled group and the multiple intoxication group, respectively, while the corresponding intoxication levels were 4.3 ± 0.3 and 4.5 ± 0.3 respectively.

At the time of electrode implantation, body weight was 300 ± 3, 296 ± 2, 294 ± 2, and 296 ± 2 g in the control group, the alcohol-withdrawal kindled group, the multiple intoxication group, and the diazepam group, respectively. Five animals from the four groups were excluded due to electrode failure, and one animal was excluded due to electrode misplacement yielding six to seven animals in each of the four groups. The numbers of kindling stimuli in experiment 3 are also shown in Table 2. This variable did not differ significantly between the four groups. As the design in experiments 2 and 3 was almost identical, we considered it reasonable to pool the data from the control group and the two alcohol-treated groups in order to increase the power of the statistical analysis (Table 2). No significant difference in the number of kindling stimuli between the three groups was found. The median (and range) of the after-discharge threshold was 100 (100–150), 100 (100–200), 150 (100–150), and 100 (100–150) µA in the control group, the alcohol-withdrawal kindled group, the multiple intoxication group, and the diazepam group respectively. These values did not differ significantly across groups (Kruskal–Wallis; $P = 0.22$). The median (and range) of the after-discharge threshold in the control group, the alcohol-withdrawal kindled group and the multiple intoxication group after the data from the second and third experiment were pooled were 100 (100–350), 100 (100–500), and 100 (100–150) µA respectively. No significant group effect was detected (Kruskal–Wallis; $P = 0.91$).

**DISCUSSION**

In the first experiment, the number of stimuli necessary to kindle the alcohol-withdrawal kindled group with seizures was significantly decreased compared with both the alcohol-withdrawal kindled group without seizures and the single episode group. The kindling rate in the control group did not differ significantly from any of the other three groups. We did, however, find a trend towards a difference between the control group and the alcohol-withdrawal kindled group
with seizures as the number of kindling stimuli was decreased by more than 50% in the latter group. Bearing in mind that the only difference between the control group and the single-episode group was that the latter group was exposed to a single episode of alcohol intoxication and alcohol withdrawal, we have tentatively concluded that the true number of amygdala stimuli needed to kindle the alcohol-withdrawal kindled group with seizures was decreased compared to the other three groups. This conclusion is in agreement with the results of Pinel et al. (1975) showing an increased alcohol-withdrawal reaction in amygdala-kindled rats. Thus, the results of the current study suggest cross-sensitivity between electrical and alcohol-withdrawal kindling, but more studies are clearly needed to confirm this hypothesis.

Although the increased kindling sensitivity in the alcohol-withdrawal kindled group with seizures in the first experiment may suggest that electrical kindling and alcohol-withdrawal kindling share common mechanisms of origin, other explanations should be considered. For instance, we cannot rule out that the increased kindling rate in this group was simply due to the previous seizures which these animals developed, although recent results from our laboratory showing that amygdala kindling was slightly retarded in rats previously exposed to multiple convulsions induced by external electrical stimulation are inconsistent with this interpretation (Kragh et al., 1993). As expected, there was a high mortality rate in all three experiments (i.e. 27–54%). This is an inevitable consequence of the high alcohol intoxication level which is necessary to produce alcohol-withdrawal symptoms following a relatively short intoxication period. Although the mortality rate per episode was low compared to the original study by Majchrowicz (1975) in which 20% of the animals died after 4 days of alcohol intoxication, we need to consider whether the animals surviving the multiple intoxication episodes are a selected group as far as amygdala kindling is concerned. Two observations speak against this speculation. First, the two alcohol-withdrawal kindled groups in experiment 1 were exposed to exactly the same alcohol regimen and therefore the significant difference in the electrical kindling rate between these two groups cannot be explained by a selection phenomenon. Second, if the animals surviving the multiple alcohol intoxication episodes represented a selected group with a different kindling sensitivity, we would have expected that the multiple intoxication groups in experiments 2 and 3 should have kindled at a different rate than the control group and the diazepam group (experiment 3 only), which they did not.

McCown and Breese (1990) used the liquid diet technique to establish chronic alcohol intoxication. They exposed rats to four cycles each consisting of 12 days of chronic alcohol intoxication and 1 day of alcohol withdrawal. In disagreement with the results of the current investigation, McCown and Breese (1990) reported that the number of stimulations necessary to kindle the amygdala in the alcohol-treated rats was increased. As the animals in this study neither developed clinical nor EEG signs of neuronal hyperactivity during the withdrawal episodes, the results of McCown and Breese (1990) raise the question as to whether multiple alcohol intoxication episodes per se can retard electrical kindling in the amygdala. Theoretically, the alcohol intoxication and the withdrawal episodes in the present investigation could have had opposite effects on the kindling sensitivity, i.e. retard and facilitate amygdala kindling, respectively. If these putative effects were of a similar magnitude they would outbalance each other resulting in an overall unaltered kindling rate, as we detected in some of the alcohol-withdrawal kindled groups in the current study. Including a group which, due to diazepam treatment, did not develop clinical alcohol-withdrawal reactions after the multiple alcohol intoxication episodes (the multiple intoxication group) allowed us to discriminate between the separate effects of alcohol intoxication and alcohol withdrawal on the number of kindling stimuli in the amygdala. Since the kindling rate was unchanged in both the multiple intoxication group and in the group which only received diazepam, the results of the present investigation disagree with the theory stated above that repeated alcohol intoxication episodes per se may retard amygdala kindling.

In addition to a retarded kindling rate in amygdala, McCown and Breese (1990) also reported a facilitation of electrical kindling in the inferior colliculus. The limited number of studies investigating cross-sensitivity between electrical kindling and alcohol-withdrawal kind-
ling make it difficult to explain the contradictory findings of McCown and Breese (1990), but the results nevertheless suggest that kindled-alcohol withdrawal behaviour is accompanied by increased neuroexcitability in some brain regions, whereas the neuroexcitability in other regions may be decreased perhaps as an adaptive response to an overall rise in cerebral hyperactivity.

In addition to the work of McCown and Breese (1990), the effect of alcohol-withdrawal kindling on subsequent electrical amygdala kindling has been studied in one previous investigation (Carrington et al., 1984). In this study, rats were exposed to seven episodes each consisting of 3 days of alcohol intoxication and 1 day of alcohol withdrawal. The repeated exposure to alcohol intoxication and withdrawal did not affect the electrical kindling rate of these animals. Carrington et al. (1984) did not monitor the severity of the withdrawal reactions. It is tempting to speculate that the convulsive withdrawal behaviour of the animals did not increase during the course of the repeated alcohol-withdrawal episodes, especially since it has not been documented that the alcohol paradigm used by Carrington et al., (1984) actually led to an augmented withdrawal seizure activity. In that case, the results of Carrington et al. (1984) are consistent with the unchanged rate of amygdala kindling in the alcohol-withdrawal kindled group without seizures in the first experiment of the present study.

As the $P$-value of the ANOVA in the second experiment was close to the chosen significance level of 0.05 (i.e. $P = 0.053$), no firm conclusions could be made from this experiment. The post-hoc test suggested that the number of kindling stimuli in the alcohol-withdrawal kindled group was decreased, compared with the control group, but we were unable to reproduce these findings in the third experiment, and when the data from experiment 2 and experiment 3 were pooled, no difference between the control group, the alcohol-withdrawal kindled group and the multiple intoxication group was found. Thus taken together, these two experiments showed that neither repetition of alcohol intoxication and alcohol withdrawal nor repetition of alcohol intoxication per se changed the electrical kindling susceptibility in the amygdala. As the seizure activity was not monitored in experiment 2 and experiment 3, the alcohol-withdrawal kindled groups in these experiments should be regarded as a mixture of two subpopulations corresponding to the alcohol-withdrawal kindled group with seizures and the alcohol-withdrawal kindled group without seizures in experiment 1, respectively. In the present alcohol-withdrawal kindling paradigm, we normally detect seizure activity in 20–25% of the animals (Ulrichsen et al., 1992, 1995). Given the results of the first experiment in which the kindling rate in the amygdala exclusively was altered in the seizure animals, we would only expect a minor decrease in the kindling rate of the alcohol-withdrawal kindled group in the second and third experiments (corresponding to 20–25% of the decrease in the number of kindling stimuli observed in the seizure animals in experiment 1). If we additionally take into account that the proportion of seizure animals may vary considerably in a relatively small sample of alcohol-withdrawal kindled animals as in the present experiment (i.e. $n = 13$), it is very difficult to compare the results of the first experiment with those of the second and third experiments. Thus the pooled results of the second and third experiments showing no alterations in the kindling rate in the amygdala in the alcohol-withdrawal kindled group are neither supportive nor in disagreement with the results of the first experiment.

Using a similar design as in experiment 1, we have performed a series of investigations in order to study whether cerebral alterations in membrane lipids (Ulrichsen et al., 1991) and various receptor systems (Ulrichsen et al., 1988, 1996, 1997) play a role in alcohol-withdrawal kindling. Unfortunately this approach is very time- and work-consuming and, as only 20–25% of the animals develop seizures, the seizure group is normally quite small making it difficult to detect subtle but potentially important biochemical alterations. In addition, if a multiple intoxication group receiving diazepam during the withdrawal reactions was to be included, as we did in experiment 2 and experiment 3 of the current study, the workload would be more or less doubled. If we had detected a change in the electrical kindling rate in the alcohol-withdrawal kindled group in these two experiments, it would have been reasonable to use the same design in future biochemical experiments. This would reduce the time and labour
necessary to perform an alcohol-withdrawal kindling experiment considerably and could ensure much larger experimental groups as compared to using the design used in experiment 1. However, the facilitated amygdala kindling in the alcohol-withdrawal kindled group with seizures in the first experiment and the negative results in experiment 2 and experiment 3 indicate that the alcohol-withdrawal kindled animals in future experiments should be differentiated by whether or not they develop spontaneous withdrawal seizures.

The electrical kindling procedure in the first experiment was slightly different from the second and third experiments. We used a constant current of 200 µA in experiment 1, while in experiments 2 and 3 we used a lower current, i.e., the current which at the first stimulation corresponded to the after-discharge threshold in the individual animal. In addition, the animals in experiment 1 were considered fully kindled when one grade 5 seizure was observed, whereas three grade 5 seizures were required in the second and third experiments. These differences explain why the number of stimuli necessary to kindle the control animals in the first experiment was considerably lower than in the second and third experiments. By changing the electrical kindling procedure in experiment 2 and experiment 3, we hoped to decrease the variability of the data, which, however, did not occur, as the standard errors of the means in these experiments were increased compared with the first experiment.

No differences in the non-convulsive withdrawal reaction were found between the three experimental groups in experiment 1. This finding is in agreement with Ulrichsen et al. (1992, 1995) and indicates that it is only the convulsive component of the withdrawal reaction which is augmented during alcohol-withdrawal kindling.

In conclusion, the present study showed that amygdala kindling was facilitated in alcohol-withdrawal kindled rats which developed seizures, compared with alcohol-withdrawal kindled animals which did not show convulsive activity and animals exposed to a single episode of alcohol intoxication and alcohol withdrawal. No firm conclusions could be drawn regarding the kindling rate in the control group and more studies are therefore needed to elucidate whether alcohol-withdrawal kindling and electrical kindling have cerebral mechanisms in common.

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