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Effects of Alcohol on Visually Evoked Responses in Rats During Addiction and Withdrawal[1]

REX A. BIERLEY, DALE S. CANNON, CHRIS K. WEHL AND ROBERT E. DUSTMAN[2]

Veterans Administration Medical Center, Salt Lake City, Utah

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BIERLEY, R. A., D. S. CANNON, C. K. WEHL AND R. E. DUSTMAN. *Effects of alcohol on visually evoked responses in rats during addiction and withdrawal.* PHARMAC. BIOCHEM. BEHAV. 12(6) 909–915, 1980.—The time course of visually evoked responses (VER's) recorded at the cortex were studied in rats during addiction and withdrawal. Data for peak-to-peak amplitudes, latencies and photic afterdischarges (PhAD's) were collected on the fifth, eleventh and seventeenth days of addiction, on the day of withdrawal and on the first, second and seventh days postwithdrawal. For the ethanol group during the addiction phase, amplitude and PhAD measures were depressed at the first recording session (day 5) and remained so throughout the addiction phase. Latency data, however, revealed a progressive increase to major peaks throughout the addicition phase that reached significance for P2 on day 17. VER amplitudes and PhAD excursion values were depressed and latencies increased, relative to controls, at the end of the addiction phase. On the day of withdrawal (day 0), P3-N3 amplitudes and PhAD excursion values remained significantly depressed relative to controls. Amplitude measures for other individual components had returned to control levels but had not yet exhibited rebound (neural hyperexcitability). Latency measures remained unchanged during day 0 testing and demonstrated a recovery to control levels by day 1 or 2 postwithdrawal. On day 1 postwithdrawal, amplitude and PhAD measures reflected neural hyperexcitability that remained throughout the seventh day although the decline in amplitudes between the second and seventh day of postwithdrawal testing suggested a trend toward neural recovery. The time course of VER amplitudes and latencies and the PhAD during addiction and withdrawal are discussed.

Alcohol Ethanol (EtOH) Addiction (chronic) Withdrawal Visual evoked response Peak-to-peak amplitudes Latencies Photic afterdischarge Recovery time Neural hyperexcitability Rebound Rats

THE ethanol (EtOH) withdrawal syndrome is considered to reflect the release of a latent state of neural excitability developed as a result of the depressant effects of continuous exposure to EtOH [17]. Neural hyperexcitability during acute withdrawal in rats has been reflected by increased amplitudes of visual evoked responses (VER's) [4,23] as well as by the progressive development of epileptiform abnormalities in spontaneous EEG activity [14,28]. Similar findings have been reported for humans [5,6], cats [22] and mice [29]. The behavioral manifestations of the EtOH withdrawal syndrome in rats fall along a continuum from hyperexcitability and piloerection to increased susceptibility to audiogenic seizures [1, 2, 8, 15].

Porjesz, Begleiter and Hurowitz [23] showed that VER amplitudes increased in withdrawing rats beginning at 6.5 hr after the last EtOH dose and remained elevated through the eighth hour, at which time testing was discontinued. Two weeks later VER amplitudes had returned to control levels. In another study, Begleiter and Coltrera [4] showed that VER amplitudes were elevated 24 hr postwithdrawal. Other time periods were not sampled.

The literature on the longterm course of neural hyperexcitability following alcohol withdrawal is inconsistent, perhaps as a result of the different methods employed. Behavioral measures, especially seizure susceptibility to electroconvulsive shock (ECS) [19], hippocampal stimulation [12], pentylenetetrozole (PTZ) [13] and auditory stimulation [11], have been used to assess neurohyperexcitability. Geisler, Hunter and Walker [12], using hippocampal stimulation to induce behavioral convulsions, reported neural hyperexcitability at 8 and 72 hr but not at 24 hr and 1 wk with the data at 24 hr being somewhat equivocal. Hunt [13], using the PTZ method, demonstrated reduced seizure thresholds on the day of withdrawal but elevations (hypoexcitability) for 1 day to 2 wk after withdrawal.

While the studies of Porjesz *et al.* [23] and Begleiter and Coltrera [4], taken together, suggest a longterm recovery time for VER amplitudes of approximately two weeks, little research has specifically addressed the issue of longterm recovery time following withdrawal from EtOH. Chu, Squires

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[2]Send reprint requests to Dr. Dale Cannon, Psychology Service, VA Medical Center, 4500 So Lancaster Rd. Dallas, TX 75216

and Starr [9], using rats, reported shorter latencies for two of the seven major peaks for far field or brain stem auditory evoked responses (BAER's) during withdrawal that persisted as long as 3–5 weeks after the last EtOH dosage but were normal by 8 weeks. In that study, they failed to obtain the increase in evoked response amplitude (rebound) generally observed following EtOH withdrawal. Since the brief (7–10 msec) far field BAER is thought to reflect primary sensory pathways in the brain stem, it may be that their results cannot be compared with evoked response data collected at cortical sites.

Thus, there are no studies that have looked specifically at the longterm recovery time of cortical evoked response activity following withdrawal from EtOH. The present experiment was designed to study the addiction-withdrawal cycle of VER's in rats at different times relative to the time of EtOH withdrawal. Also, because it has recently been demonstrated that the photically evoked afterdischarge (PhAD) that follows the VER is sensitive to pharmacological modulation [25], PhAD's were recorded to determine their relationship to EtOH intoxication and withdrawal.

METHOD

Subjects

Eighteen 120-day-old Long-Evans rats with a mean initial weight of 370 g served as subjects. All rats were housed individually in standard wire mesh cages for the duration of the experiment. Two cohorts of animals were run to facilitate testing of all subjects during withdrawal. The first cohort contained six experimental and four control subjects, and the second cohort contained five experimental and three control subjects. Throughout the study, the experimental treatment and collection of data for the two cohorts were staggered by one week.

Apparatus

All electrophysiological recording was conducted in a semi-darkened and electrically shielded recording room. A Grass Model PS22 photostimulator, set to direct 10 μsec light pulses into a reflecting hemicylinder, provided constant stimulation regardless of the animal's head position. The illumination of the reflecting surface was 20 lux as measured from the position of the rat's eyes. The hemicylinder was placed in front of a hammock in which the animals were held under light restraint. Background white noise at 70 db with reference to 0.0002 μ bars was fed into the recording room to mask extraneous sounds. The EEG was amplified by a Grass Model 78B EEG/polygraph (bandwidth, 0.3–300 Hz; time constant, 0.25 sec) and recorded on magnetic tape. The stored EEG was digitized at a rate of 500/sec and fed into a Terak computer (DEC LSI-11 based) which summed and averaged VER's and PhAD's. These responses were traced on graph paper by a Complot DP-1 plotter.

Electrophysiological recordings were made following 15 min of iterative stimulation. Photic afterdischarges were elicited by photic pulses presented at a rate of 1 pulse/7 sec. This rate has been determined as optimal for the elicitation of PhAD's [25]. The VER's were recorded to photic stimulation manually delevered at a rate of approximately 1 pulse/2–3 sec. This rate generally precludes the generation of PhAD's following the VER [25]. Twenty-five responses from the right visual cortex during periods free from movement artifact were recorded for computer averaging. The VER generally consisted of three positive-negative going components. At the 1 pulse/7 sec presentation rate, the VER was typically followed by the rhythmic PhAD [26]. In order to gain an indication of changes in the PhAD due to EtOH intoxication and withdrawal, total excursion of the PhAD tracing was measured from the third positive wave (P3) of the VER to the end of each 1 sec plot with a map reading wheel. In addition to the PhAD excursion values, peak-to-peak amplitudes and peak latencies of the VER wave were examined for systamatic effects due to EtOH intoxication and withdrawal. For ease of comparison with individual amplitude components, a combined amplitude measure is provided. This measure, showing the general pattern of the VER in response to EtOH addiction and withdrawal, was obtained by direct addition of the five major components.

Procedure

Rats were food deprived for 8–12 hr prior to surgery and injected with atropine sulfate (0.2 cc IP) just prior to the surgical procedure. Rats were anesthetized with sodium pentabarbital (45 mg/kg) and surgically prepared with indwelling extradural stainless steel screw electrodes implanted over right and left visual cortices at points 7 mm posterior to bregma and 3 mm lateral to the midline suture. Two more screw electrodes were implanted in the calvarium overlying the cerebellum and frontal sinus area for reference and grounding respectively. Lead wires were attached to a 4-pin electrode pedestal fabricated from Amphenol miniature connectors. Ten days to two weeks were allowed for animals to recover from surgery.

Rats were then reduced to 75% of their ad lib weights over a 14 day period by allowing them access to approximately 5 g of Purina Lab Chow each day. The addiction phase began on the fifteenth day after food deprivation was initiated. Food and water were withdrawn from all animals and replaced with a liquid diet consisting of Sustacal (Mead-Johnson & Co.) fortified with either EtOH or sucrose. The liquid diet was presented in inverted 100 ml graduated cylinders to allow determination of liquid consumption without bottle removal. Consumption was recorded at twenty-four hr intervals throughout the twenty-one days of the addiction phase. The EtOH group received the liquid diet with 40% of its calories derived from EtOH. The EtOH-Sustacal diet was prepared by mixing 131.5 ml EtOH/liter Sustacal (11.5% v/v). Previous research [2,15] suggests that rats will drink sufficient quantities of the EtOH-Sustacal diet to produce EtOH dependence while receiving 100% of the daily nutrient requirements for the rat [30] and maintaining constant weight gain. The control group received a calorically equivalent Sustacal-sucrose diet in quantities yoked to the average for the EtOH group. The diet for the control group was prepared by adding enough tap water to 212 g sucrose to make a 244 ml sucrose solution and adding this mixture to one liter of Sustacal. Concentrations of EtOH and sucrose in Sustacal remained the same throughout the addiction phase.

Animals were habituated to the restraining harness on days 2, 5, 8, 11, 15 and 17 of the addiction phase by placing the animal in the harness for 15 min of photic stimulation. Baseline PhAD's and VER's were recorded after the 15 min habituation period on days 5, 11 and 17. After twenty-one days of liquid diet presentation, these solutions were removed. Visual evoked responses and PhAD's were recorded 8 hr postwithdrawal (day 0 withdrawal) when behavioral signs of withdrawal were greatest. Animals were then re-

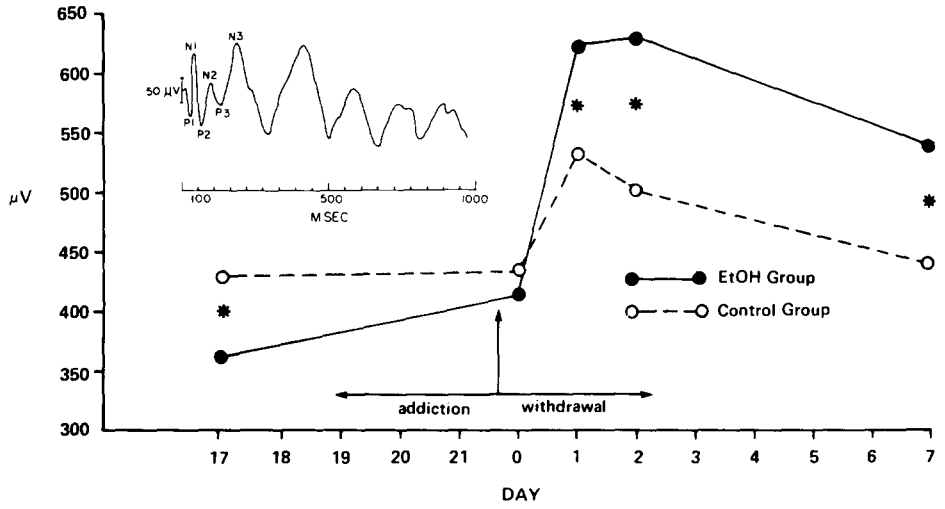

FIG. 1. Combined amplitudes during EtOH addiction and withdrawal. Vertical arrow indicates point at which EtOH was withdrawn. Day 0 values represent VER testing 8 hr postwithdrawal. Asterisks indicate those days on which groups differed significantly. Inset is an example of a typical visual evoked response followed by a photic afterdischarge.

turned to ad lib water and laboratory chow for the duration of the experiment. Additional VER's and PhAD's were recorded on the first, second and seventh days postwithdrawal.

RESULTS

One animal in the EtOH group developed spontaneous convulsions on the day of withdrawal prior to electrophysiological testing and was eliminated from the study.

Body weights for the EtOH and control groups were compared on the first and last days of the addiction phase. Two-tailed dependent t-tests revealed no differences at either time. The average daily EtOH-dose for the experimental group was 12.7 g/kg/day.

Multiple bivariate split-plot analyses of variance with repeated measures across day 17 of addiction and days 0, 1, 2 and 7 of withdrawal were conducted on the amplitude, latency and PhAD measures [28]. Heterogeneity of population-error variance across repeated measures was tested using Hartley's F_{max} test. This analysis was applied in each case where significant Days or Group × Days interaction effects were obtained. None of the F_{max} tests reached statistical significance (all $p>0.05$) obviating the need for the Geisser-Greenhouse correction for non-homogeneous error variances across repeated measures [31]. Where the split-plot analysis yielded reliable Group × Days interactions, simple main effects tests between groups were conducted to determine specific days for which the group means differed significantly [31]. Significant between-group differences for individual days are indicated on each figure by asterisks. Since the error term for this simple main effects test represents a pooling of what is often heterogeneous sources of variance, Satterthwaite's correction [31] was used when necessary.

Split-plot analysis of the combined amplitudes data (cf. Fig. 1) revealed a significant Group × Days interaction, $F(4,60)=17.06$, $p<0.001$. Simple main effects tests between the two groups yielded significant results for all days except the day of withdrawal (day 0). These differences, reflect the typical depression of VER amplitudes during intoxication

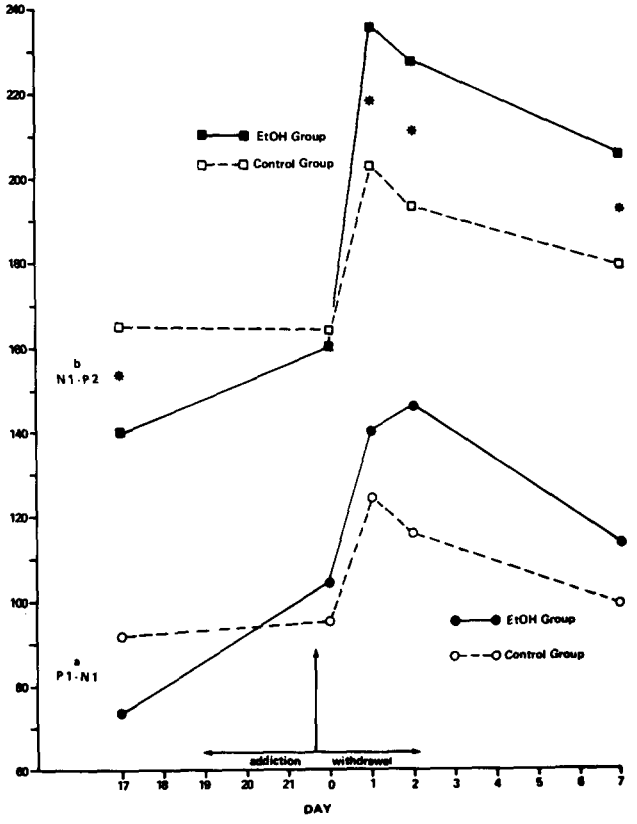

FIG. 2. Amplitudes for two individual components during EtOH addiction and withdrawal. Vertical arrow indicates point at which EtOH was withdrawn. Day 0 values represent VER testing 8 hr postwithdrawal. Asterisks indicate those days on which groups differed significantly.

(day 17 addiction) and the enhancement of VER amplitudes (rebound) following EtOH withdrawal (days 1, 2 and 7 postwithdrawal).

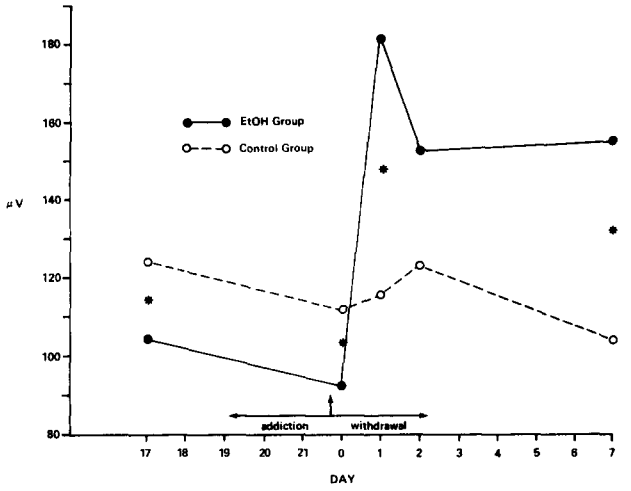

FIG. 3. Amplitude measure for individual component P3-N3 during EtOH addiction and withdrawal. Vertical arrow indicates point at which EtOH was withdrawn. Day 0 values represent VER testing 8 hr postwithdrawal. Asterisks indicate those days on which groups differed significantly.

Analysis of individual components indicated that P1-N1, N1-P2 and P3-N3 best accounted for the pattern seen in Fig. 1. N1-P2 (cf. Fig. 2b), which appeared to be the major contributor to this pattern, yielded a significant Group × Days interaction, F (4,60)=14.53, $p<0.001$. Tests for between-groups simple main effects were identical to those for the combined amplitude data as indicated in Fig. 2b. The amplitude data for P1-N1 (cf. Fig. 2a) appeared to follow a similar pattern, but the Group × Days interaction failed to reach significance, F (4,60)=2.12, $p=0.09$, so that simple main effects could not be tested.

Analysis of the P3-N3 component revealed a significant Group × Days interaction, F (4,60)=5.78, $p<0.001$. Subsequent analysis for simple main effects between groups revealed results similar to these for P1-N1 and N1-P2 with one notable exception. The P3-N3 amplitude which was depressed during intoxication (day 17 addiction) remained depressed, relative to the control group, during the early period of withdrawal (day 0) before showing the typical rebound on days 1, 2 and 7 postwithdrawal (cf. Fig. 3). Main effects for P2-N2 and N2-P3 were not significant.

Latency data for the six major peaks were subjected to the same split-plot analysis of variance. Latencies to all peaks were longer for the EtOH group during intoxication (day 17 addiction) and generally showed a decline to, rather than a rebound below, control levels during the postwithdrawal testing period. This pattern, which was not statistically significant for the late components (occurring after about 50 msec) was reasonably consistent for the earlier peaks. The data were best typified by P2 latencies (cf. Fig. 4b) which yielded significant Group, F (1,15)=8.16, $p<0.05$, and Group × Days interaction, F (4,60)=2.60, $p<0.05$, effects. Simple main effects tests between groups revealed this difference to be significant during intoxication (day 17 addiction) and postwithdrawal for days 0 and 1. By the second day postwithdrawal, latencies for the EtOH group were no longer statistically different from those of the control group indicating some recovery from the hyperexcited neural state that accompanies EtOH withdrawal.

A small but statistically significant Group difference was also found for P1 latencies, F (1,15)=7.38, $p<0.05$, but the Group × Days interaction was not significant so that simple main effects tests between groups were not justified. However, the P1 latencies displayed a trend that paralleled those for P2. There was a significant Group × Days interaciton for N1 latencies, F (4,60)=3.60, $p<0.05$. Simple main effects tests using Satterthwaite's correction for non-homogeneous sources of error variance indicated that N1 latencies were significantly longer during intoxication (day 17 addiction)

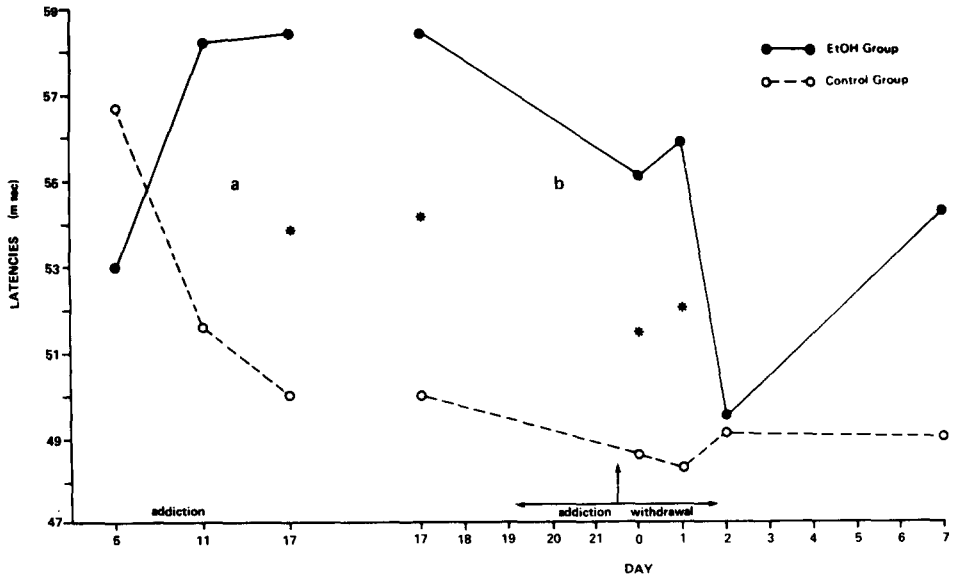

FIG. 4. Latencies to VER peak P2 during EtOH addiction and withdrawal. Part a indicates the time course of latencies during the addiction phase. Part b shows the time course of latencies relative to the point at which EtOH was withdrawn (vertical arrow). Day 0 values represent VER testing 8 hr postwithdrawal. Asterisks indicate those days on which groups differed significantly.

and on the day of withdrawal (day 0). This group difference was no longer significant at 1 day postwithdrawal and thereafter. Main effects for the late components were not significant.

Latency, amplitude and PhAD data were also analyzed for changes during the 21 day addiction phase. Because of technical problems at the start of the experiment, data for two animals in each group were lost on different recording sessions. These values were estimated using the group mean for the appropriate session. Analysis of the amplitude and PhAD data revealed no significant changes across days. Latencies to major peaks, however, revealed a trend toward longer latencies with increasing duration of addiction. A split-plot analysis of variance revealed a significant Group × Days interaction for P2 latencies, $F(2,30)=4.18$, $p<0.05$ (cf. Fig. 4a). Simple main effects tests using Satterthwaite's correction were conducted on the group data. As can be seen in Fig. 4a, day 5 latencies for the EtOH group were shorter relative to the control group but this difference was not significant. By day 11, when the control group had demonstrated a considerable amount of habituation to the testing procedure, the latencies for the EtOH group were much longer than for control animals but the group difference did not reach significance until day 17, $F(1,17)=4.29$, $p<0.05$.

A split-plot analysis of variance revealed no overall group differences between the EtOH and control groups for the PhAD measure. However, simple one-way analyses of variance [31] indicated an increase in excursion values during withdrawal for the EtOH group, $F(4,36)=6.58$, $p<0.01$, but not for the control group. A Scheffe's test [31] revealed that the combined pre- and early withdrawal sessions (day 17 addiction, day 0 withdrawal) differed significantly from the combined 1, 2 and 7 day postwithdrawal sessions, $F(1,36)=24.79$, $p<0.025$.

DISCUSSION

The present findings are generally consistent with reports that evoked potential amplitudes are depressed during EtOH intoxication and rebound to reflect neural hyperexcitability during withdrawal (cf. [16,18]). It has been reported that the depressant effects of EtOH are reflected in the later components of the evoked response with latencies greater than 50 msec [16] or greater than 80 msec [18]. These reports are based upon acute studies where evoked potentials were recorded following a single intoxicating dose of EtOH. However, Begleiter, Branchey and Kissen [3] have shown that a single intoxicating dose of EtOH in rats can significantly depress the VER amplitude of early components (P1-N1 at the reticular formation, N1-P2 at the visual cortex) if the dose is large enough (1.5 g/kg). Furthermore, Begleiter and Coltrera [4] have shown that chronically intoxicated (14 day intubated) rats show rebound of early (P1-N1 at approximately 50 msec), as well as late (P2-N2 between 100–180 msec), cortical components during withdrawal indicating an effect of EtOH on these components during intoxication. It is possible that both dose and duration of EtOH consumption contribute to its effect on early evoked response components. In the present study, consistent with Begleiter and Coltrera's [4] results, both early (P1-N1, N1-P2) and late (P3-N3) components showed depression of VER amplitudes during intoxication (day 17 addiction) and postwithdrawal rebound.

During the addiction phase, VER amplitudes and PhAD excursion values for the EtOH group were already depressed on day 5 and remained so throughout the 21 days of addiction. The latency measure, however, reflected a progressive depressant effect due to EtOH consumption. Visual evoked response latencies for one early (P2) component were significantly longer on day 17 of addiction, relative to the control group, than latencies measured on days 5 and 11. Few studies have reported effects of EtOH on latency changes, which are thought to be more resistent to experimental manipulations. Chu, Squires and Starr [9] reported increased latencies for two BAER peaks during chronic (14 day) intoxication and decreased latencies to six of the seven peaks during withdrawal. Comparisons between groups in the present study indicate that P2 latencies were significantly longer during intoxication (day 17 addiction) but failed to show a rebound postwithdrawal as demonstrated by Chu, Squires and Starr [9]. Their results cannot be directly compared because the BAER is relatively brief (7–10 msec) and was evoked via a different modality. Moreover, they did not find amplitude changes, suggesting that the BAER and cortical VER may be fundamentally different in their response to EtOH.

In the present study, VER amplitude was still significantly greater for the EtOH group at seven days postwithdrawal. However, the decline of the amplitude measure toward control levels suggests that the evoked potential amplitude was returning to normal levels. This decline suggests a possible recovery time for amplitudes consistent with the two week period observed by Porjesz, Begleiter and Hurowitz [23]. For the latency measures there was no rebound after withdrawal and a more rapid recovery to control levels in comparison to the amplitude measures. This may be related to the time course of EtOH's effects during intoxication. While the amplitude measure for the EtOH group was already depressed during the first recording session (day 5 addiction) and remained depressed throughout the addiction phase, the latency measure showed a progressive increase in peak latencies in response to chronic intoxication that did not reach significance until the seventeenth day of addiction. Therefore, it is suggested that (a) major peak latencies are more resistant to EtOH's effect and also that (b) recovery is more rapid due to the relatively short period (days 17–21 of addiction) during which EtOH exerted a significant effect on latencies. It should be noted that the amplitude pattern across days for VER component P3-N3 differed from that for P1-N1, N1-P2 and the combined amplitudes, but was quite similar to that of the PhAD. Both the P3-N3 amplitude and PhAD were depressed during intoxication (day 17 addiction) and early withdrawal (day 0) but rebounded on days 1, 2 and 7 postwithdrawal. Shearer (personal communication) suggests that whether or not PhAD activity follows the VER at non-optimal rates of photic stimulation (e.g., that used for VER testing, 1 pulse/2–3 sec) depends on the degree to which the animal has been habituated. If the amount of habituation is sufficient, PhAD's will occassionally follow the VER and will be reflected in the P3-N3 component. In the present study, animals were extensively habituated (10 sessions, 15–20 min stimulation pre-PhAD testing) accounting for the similar pattern across days for P3-N3 and the PhAD. Consistent with this interpretation,Schwartzbaum [24] has postulated that the late negative wave (P3-N3) and PhAD's have a similar neurogenesis. However, the possibility that the PhAD elaboration is generated by a different neural system than that for P3-N3 has been discussed by Bigler [7].

The PhAD has been shown to depend on an optimal level

of arousal since either hypo- or hyper-arousal depresses the PhAD [25]. It is therefore possible that the P3-N3 amplitude and PhAD excursion values, which are depressed on day 17 addiction due to intoxication, remain depressed on day 0 withdrawal due to hyperarousal produced by increasing neural activity following EtOH withdrawal. If this were the case, it might suggest that the PhAD excursion and P3-N3 amplitude measures are more sensitive indices of withdrawal because they reflect neural hyperexcitability earlier than do the amplitude measures for the remaining individual components. However, the PhAD and P3-N3 rebound on day 1 postwithdrawal when the remaining individual amplitude and latency measures indicate a continued state of hyperarousal. This suggests that the PhAD excursion and P3-N3 amplitude measures either do not respond to level of arousal in any simple fashion or that the PhAD and P3-N3 follow a different time course during the withdrawal syndrome.

There has been some concern that experimental outcomes of EtOH addiction studies are confounded by an interaction between nutrition-deficiency and EtOH consumption [2, 20, 21]. Use of the nutritionally complete Sustacal-EtOH diet in the present study produced alcohol dependent rats without weight loss. An isocalorically equivalent Sustacal-sucrose solution was presented to the control group in quantities yoked to consumption by the EtOH group. Analysis of body weights revealed that both groups gained weight over the course of the addiction phase and, further, that group weights did not differ significantly either at the start or end of the experiment.

It may be noted that the VER amplitude measure for control animals showed a shift (not statistically significant) following the day of withdrawal with a return to original levels by day 7 postwithdrawal. The fact that the baseline remained relatively more stable for the latency measures suggests that the amplitude measure is more sensitive to procedural variables. Two events occured on the day of withdrawal that might account for the amplitude shift observed on days 1 and 2 postwithdrawal. First, animals were switched from the Sustacal diet to standard lab chow and water. Secondly, prior to the day of withdrawal, either VER recordings or habituation sessions had been conducted every three days. It is possible that the change in diet and/or stress induced by daily VER recording sessions accounts for the shift in VER amplitudes for the control animals. It is important to note that the EtOH group displays a parallel shift in VER amplitudes such that the differences between groups can be attributed to withdrawal symptomatology and not to spurious fluctuations in control levels.

SUMMARY

The present results are reasonably consistent with reports that evoked potential amplitudes are depressed during intoxication and enhanced during withdrawal. In addition, our results suggest that duration of chronic intoxication may contribute to EtOH's effect on both amplitude and latency of evoked potentials, especially for the early components (those earlier than approximately 50 msec). The relatively more rapid recovery of the latency measure following withdrawal may be related to both a stronger resistence to EtOH and to the shorter period of time (days 17–21 addiction) during which EtOH exerted a significant influence.

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