In: Gross, M.M. (ed.),
Advances in experimental medicine and biology, Volume 85B,
Alcohol intoxication and withdrawal - IIIb:
Studies in Alcohol dependence,
Plenum Publishing Corporation, New York, 209-222

PERSISTENCE OF BRAIN HYPEREXCITABILITY FOLLOWING CHRONIC ALCOHOL EXPOSURE IN RATS

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ABSTRACT

Eighteen hooded Long-Evans rats were implanted with monopolar electrodes for the purpose of recording visual evoked potentials (VEP's) at the following brain sites: visual cortex, reticular formation and thalamus. Baseline VEP's were obtained to flashes for all animals, and subsequently twelve rats were intubated daily with a progression of increasing quantities of 20% (V/V) alcohol (3-8 g/kg), while the remaining six rats received an equivalent amount of water in the same fashion. Beginning 4½ hours after the last dose of intubated alcohol, withdrawal VEP's were sampled every half-hour up to 8 hours, and 24-27 hours post-withdrawal. All experimental animals manifested their greatest brain hyperexcitability at visual cortex, which peaked sharply between 7-8 hours after alcohol withdrawal. Following two weeks of abstinence, half of the experimental rats (N=6) and half of the controls (N=3) received an alcohol challenge dose (2 g/kg i.p.), while the remaining animals received the same challenge dose after five weeks. Marked hyperexcitability was observed in the two-week challenge dose animals that had been previously subjected to alcohol; no such increase in VEP amplitude was apparent for control rats. There is also some evidence of hyperexcitability after five weeks of abstinence from alcohol at visual cortex. The data indicates that the neurophysiological responses of post-addict rats to challenge doses of alcohol are readily distinguishable from those of naive animals, even five weeks after alcohol removal. Furthermore, alcohol seems to act differently at different sites of the brain.

This research was supported by grant NIAAA 01231

INTRODUCTION

It has been postulated that withdrawal from chronic alcohol ingestion is manifested by hyperexcitability of the central nervous system. We recently reported a study in which we investigated changes in evoked brain potentials recorded from human alcoholics during intoxication and withdrawal (Begleiter, Porjesz and Yerre-Grubstein, 1974). Somatosensory evoked potentials were recorded every morning, 10 hours after the last drink during the three days of baseline, four days of alcoholization, and four days subsequent to withdrawal from alcohol. Our results indicated a progressive increase of brain excitability starting with the intoxication period and reaching asymptote with the first day of total alcohol withdrawal. During the subsequent days of testing, the recovery function of somatosensory evoked potentials decreased, approaching the level obtained during baseline determinations. This rather prompt return of physiological function to normal values certainly appeared inconsistent with findings reported in the literature.

The reactivation of withdrawal symptoms after a short period of exposure to alcohol has been reported by Mendelson, Stein and McGuire (1966) who compared the effects of a 4-day period of alcoholization in four alcoholics and four normal subjects. Following cessation of drinking, two of the alcoholic subjects showed some withdrawal symptomatology, while none of the controls did. The authors interpreted their results as indicating that alcoholic patients have a predisposition to develop withdrawal symptoms. In similar experiments in animals in our laboratory, Branchey, Rauscher and Kissin (1971) demonstrated that the establishment of a state of physical dependence increased the incidence of withdrawal symptoms following a subsequent period of alcoholization. The administration of an alcohol diet did not induce any noticeable withdrawal symptomatology in animals not previously exposed to alcohol. On the other hand, when previously alcohol-dependent animals were subjected to the same procedure, 50% of them demonstrated severe withdrawal. These two studies were the first demonstrations that "latent" physical dependence may persist in an attenuated form long beyond the clinically observable withdrawal from alcohol.

The above findings strongly suggest that central nervous system (CNS) disturbances persist far beyond the administration and removal of ethanol. We have recently reported that brain hyperexcitability can be observed in rats 24 hours after their last dose of ethanol (Begleiter and Coltrera, 1975). We have also reported (Porjesz, Begleiter and Hurowitz, 1976) that the neurophysiological responses of post-addict rats to challenge doses of alcohol are readily distinguishable from those of naive animals.

The present study is an attempt to investigate the persistent electrophysiological changes caused by chronic alcohol intake and the reactivation of these changes by the administration of an alcohol challenge dose. In addition we attempted to study the possible CNS locus of these changes in the brains of rats.

METHODS

Eighteen male hooded Long-Evans rats, with a mean weight of 391 gms were used in this experiment. They were housed individually in stainless steel cages with continuous access to food and water during the entire study.

Stereotaxic surgery was performed under Diabutal anesthesia (0.8 cc/kg)for the purpose of recording visual evoked potentials (VEP's). Two monopolar-teflon-coated stainless steel depth electrodes were implanted in the ascending reticular formation (RF) and thalamus. Specific coordinates of the RF placement were: 4.2 mm posterior to bregma 2.2 mm lateral to the midline (left) and 7.0 mm from the surface of the brain; coordinates for the dorsomedial nucleus of the thalamus were: 3.0 mm posterior to bregma, 2.2 mm lateral to the midline, and 6.5 mm deep, according to the stereotaxic atlas of Pellegrino and Cushman (1967). Stainless steel screw electrodes were placed in the skull overlying the visual cortex, and two similar screw electrodes were placed bilaterally over the frontal sinus to serve as reference and ground. All leads were attached to a miniature connector and the assembly was fastened to the skull with acrylic cement.

The animals were allowed 1 to 2 weeks to recover from surgery, at which time they were placed in a sound-attenuated enclosure (IAC) and baseline visual evoked potentials (VEP's) were recorded. During the recording sessions, the skull pedestal was attached to a cable connected to a mercury-pool swivel, allowing the animals freedom of movement.

Photic stimulation was delivered with an Iconix stroboscopic light, set at peak intensity of 1,000 lm and duration of 5 msec., at a rate of 1/2.5 sec. for a total of 50 flashes. VEP's were amplified by a Grass Model 78 Polygraph and fed into a PDP11-40 computer for on-line signal averaging of a 500 msec. epoch. All data were stored on discs for subsequent analysis. Amplitude measures were obtained for all evoked potentials recorded at the three electrodes. Only the major early and late components of each evoked potential were measured in order to avoid subjective judgments and poor reliability.

Baseline evoked potentials were obtained for each animal individually, following a habituation procedure of at least 100 stimuli. Throughout the experiment each animal was tested on a carefully timed, staggered schedule such that only one rat was tested for baseline, withdrawal or challenge-dose recordings per day.

Beginning on the morning following baseline determinations, 12 rats were intubated daily for 14 days with a progression of increasing quantities (3-8 g/kg) of 20% (V/V) solution of 95% alcohol as follows: 3 g/kg for the first two days, 4 g/kg for the next two days, 5 g/kg for the next two days, 6 g/kg for the next two days, 7 g/kg for the following four days, and 8 g/kg for the remaining two days. Six control rats received an equivalent amount of water in the same fashion. VEP recordings were obtained beginning 4½ hours after the last intubated dose and were sampled every half-hour up to eight hours after the last intubation.

Following two weeks of abstinence, half of the experimental animals (N=6) and half of the controls (N=3) received an alcohol challenge-dose (2 g/kg 20% (V/V) of 95% ethyl alcohol) intraperitoneally (i.p.) while the remaining animals received the same challenge-dose after 5 weeks. VEP's were recorded immediately preceding the alcohol injection (baseline) and were sampled every twenty minutes following the alcohol challenge for the first two hours. Thereafter, VEP's were recorded each hour for seven hours post-injection.

RESULTS

Animals in both the experimental and control groups gained an average of 23 g during the two-week intubation period; their average weights were 382 and 380 g respectively at that time. All evoked potentials differences between experimental and control animals were assessed with the use of analysis of covariance with repeated measurements and unequal N. The covariate was the possible initial (first baseline) difference in VEP's and the repeated measures were the various time segments.

Before intubation there were no significant differences in baseline VEP's recorded at all electrode sites between the experimental and control groups (Figure 1). The only significant differences in VEP's between experimental and control groups during the first 8 hours subsequent to withdrawal from alcohol was found at visual cortex ($p < .05$ for early component and $p < .001$, late component). The maximum withdrawal effect was found at 7.5-8 hours post-withdrawal and was manifested by a marked increase

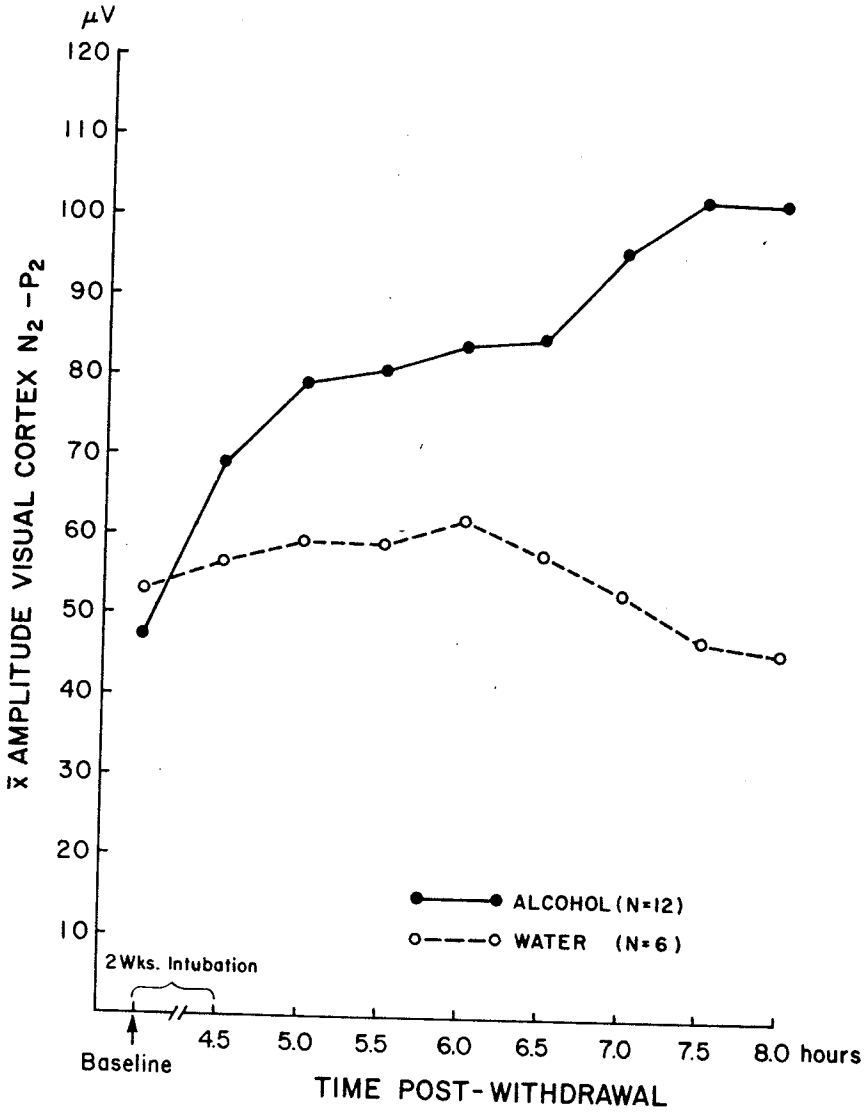

Fig. 1. Mean VEP amplitude N2-P2 recorded at visual cortex (VC) at baseline (prior to treatment) and 4.5-8 hours after two weeks daily intubation of either alcohol or water respectively.

in the late component N2-P2 of the evoked potential recorded at visual cortex.

Twenty-four hours after the last intubation, the late component visual cortex VEP differences (Figure 2) between the control and experimental animals were still significant ($p < .02$). Two weeks after the last intubation the baseline difference in VEP's between experimental and control groups was not significant, although as can be observed in Figure 3, at Time 0 (T0), the alcohol group tends to be higher. Subsequent to the injection of the challenge dose of ethanol, the difference between experimental and control groups was significant at $p < .001$ for the late component, and $p < .02$ for the early component. As can be seen in Figure 3 the maximum withdrawal effect was found at 6 hours post-injection of ethanol. This difference was significant at $p < .01$.

The experimental and control groups that were tested five weeks after intubation did not show statistically significant differences in VEP's prior to the challenge dose of ethanol. As can be seen in Figure 4, the two groups behaved quite differently after the challenge dose; however this difference was not statistically significant.

It should be noted that while our VEP's recorded at the reticular formation, and dorsomedial nucleus of the thalamus did not yield any statistically significant differences, the recordings at the thalamus indicated a rather strong trend for differences between our two groups.

DISCUSSION

Our results indicate that rats previously exposed to alcohol for a period of two weeks, show a substantial increase in central nervous system hyperexcitability during withdrawal. These findings are consistent with results obtained in mice (McQuarrie and Fingel, 1958; Freund, 1969; Goldstein, 1972; Kakihana, Butler, Hathaway and Noble, 1971; Walker and Zornetzer, 1974), in rats (Guerrero-Figueroa, Rye, Gallant and Bishop, 1970; Hunter, Boast, Walker and Zornetzer, 1973; Branchey, Rauscher and Kissin, 1971; Gibbons, Kalant, LeBlanc and Clark, 1972; Hunt, 1973; Majchrowicz, 1975; DeNoble and Begleiter, 1976), in monkeys (Ellis and Pick, 1970), and in man (Mendelson, 1964; Gross, Lewis and Hastey, 1974; Victor and Adams, 1953; Begleiter, Porjesz and Yerre-Grubstein, 1974). The data indicate that while this neural hyperexcitability can be observed at the dorsomedial nucleus of the thalamus and the ascending reticular formation, it is only statistically significant at the visual cortex. This result is consistent with our past findings (Begleiter, Branchey and Kissin, 1972; Begleiter and

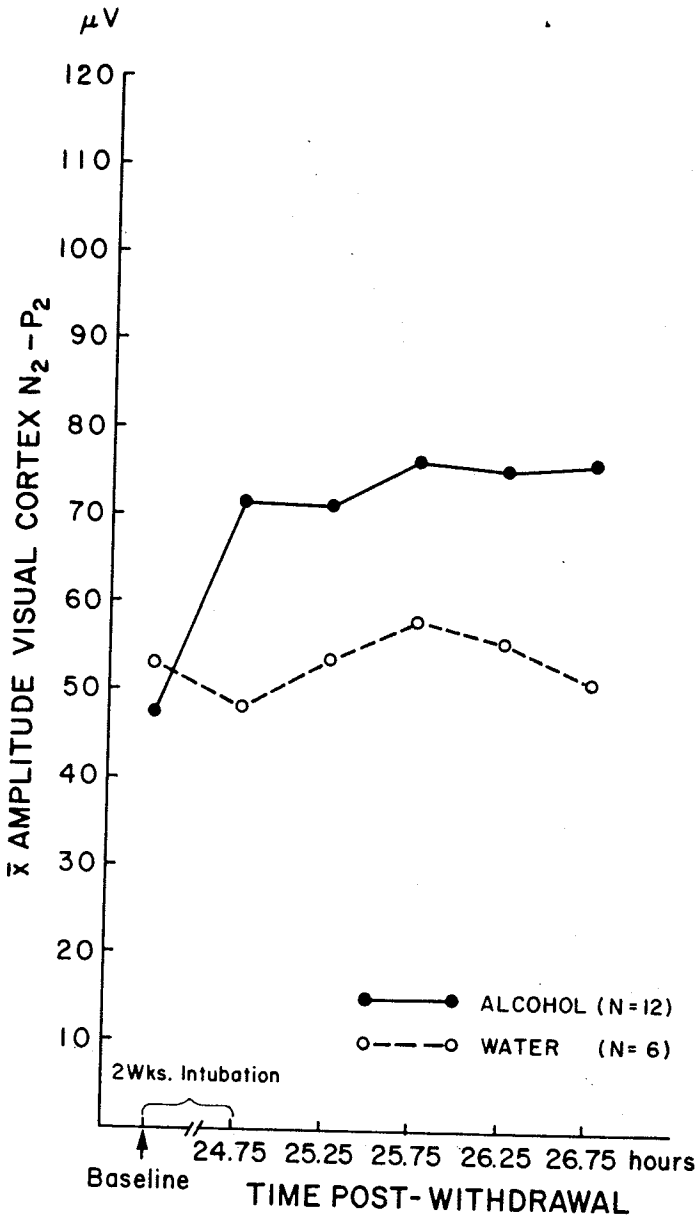

Fig. 2. Mean VEP amplitude N2-P2 recorded at visual cortex (VC) at baseline and starting at 24 hours and 45 minutes after the last intubation of either alcohol or water respectively.

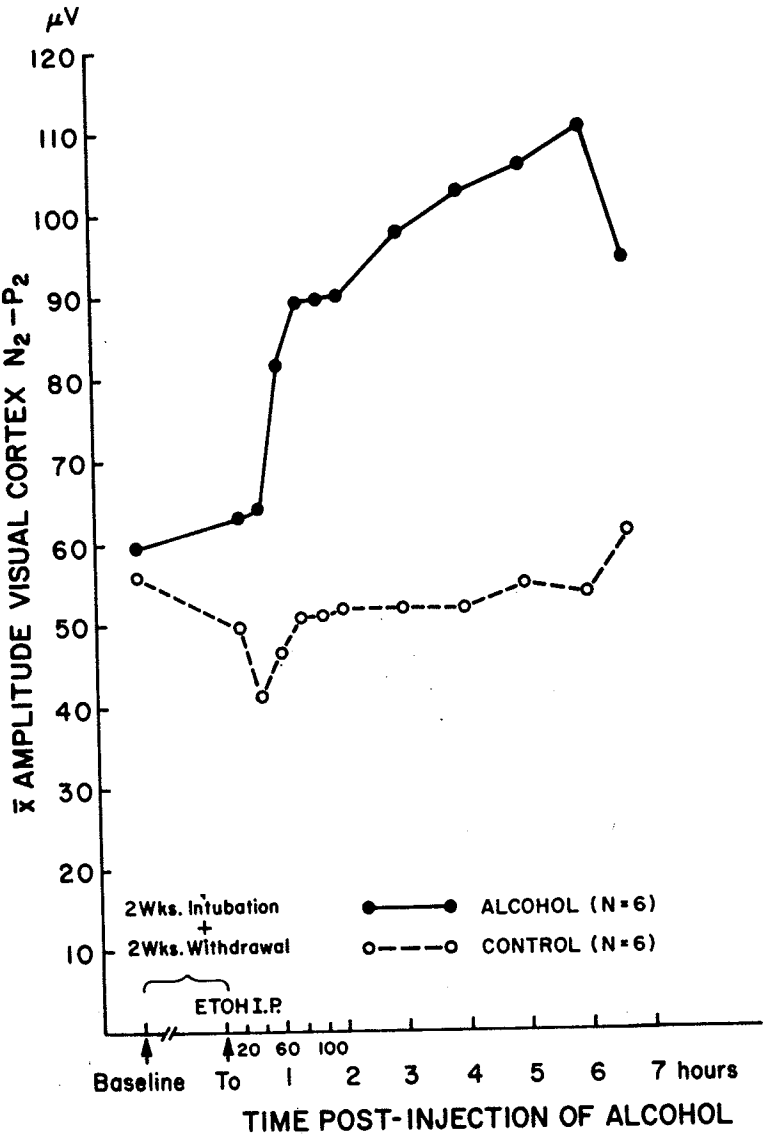

Fig. 3. Mean VEP amplitude N2-P2 recorded at visual cortex in rats receiving a challenge dose of alcohol (2 g/kg i.p.) following two weeks of abstinence from either intubated alcohol (experimental) or water (control).

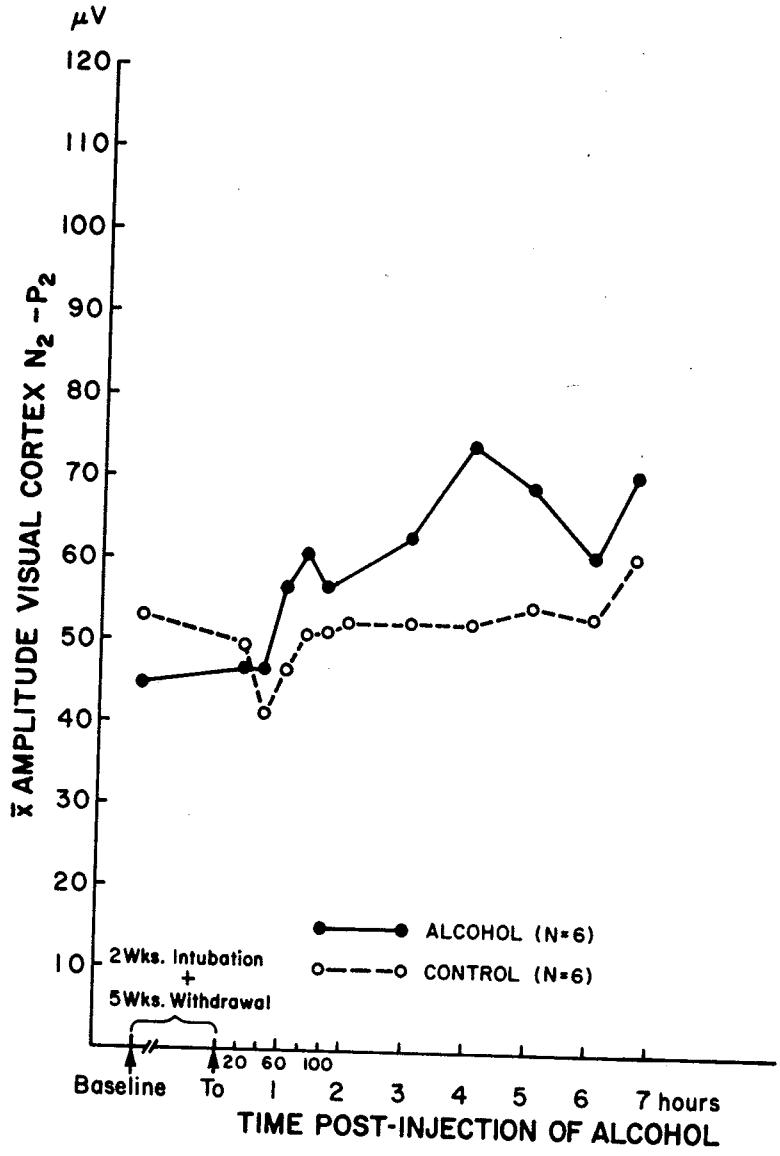

Fig. 4. Mean VEP amplitude N2-P2 recorded at visual cortex in rats receiving a challenge dose of alcohol (2 g/kg i.p.) following five weeks of abstinence from either intubated alcohol (experimental) or water (control).

Coltrera, 1976) and is also in agreement with the recent data reported by Klemm, Mallari, Dreyfus, Fiske, Forney and Mikeska, 1976. These authors recorded multiple-unit activity from 14 different brain regions in rabbits to study the location of possible "target-sites" for the action of ethanol. Their results indicate that the brain regions most sensitive to ethanol are to be found in the cerebellar and cerebral cortices.

In recent years it has become increasingly apparent that there are long-term aberrations which follow the chronic ingestion of alcohol. These long-term aberrations are often characterized by persistent dysequilibrium in physiological and psychological function which appear to reflect the presence of a "post-withdrawal syndrome." Kissin, Schenker and Schenker (1959) reported a broad spectrum of physiological imbalances in a group of hospitalized post-withdrawal alcoholics who had been abstinent for 2-3 weeks. These observations were demonstrated in autonomic nervous system, electrolyte balance, psychomotor performance, and endocrine and respiratory functions. The authors demonstrated that these aberrations could also be found in a group of alcoholics in AA abstinent from alcohol for 2-10 years, although to a lesser degree. These deficits also appear in the sleep characteristics of abstinent alcoholics.

Gross and colleagues (1973) reported that slow-wave sleep (SWS) was suppressed or totally absent in a number of abstinent alcoholics. Similar findings have been reported by Zarcone et al (1975) Johnson (1971) Wagman and Allen (1975).

Our present animal data confirm the presence of a post-withdrawal syndrome which appears to reflect an impairment of central inhibitory mechanisms resulting in increased central nervous system (CNS) excitability. It is obvious from our data that the electrophysiological responses of the experimental animals are different from those of the control animals after the administration of a challenge dose of alcohol. In response to the challenge dose of alcohol, the control animals manifest a normal depression in evoked brain potentials while the experimental animals do not show this depression but instead show a progressive increase in VEP voltage.

Our findings suggest that the biological bases of chronic alcohol intake involve complex CNS changes. These CNS changes appear to be long-lasting and can best be observed subsequent to the administration of a challenge dose of alcohol. This challenge dose of alcohol might well represent the pharmacological stimulus responsible for the retrieval of this "biological addiction-memory." These persisting CNS changes have been observed in animals by several investigators (Branchey, Rauscher and Kissin, 1971; Walker and Zornetzer, 1974; Freund and Walker, 1971; Gitlow,

Bentkover, Dziedzic and Khazan, 1973; Kakihana, Butler, Hathaway and Noble, 1971; Gitlow, Dziedzic and Dziedzic, this symposium; Porjesz, Begleiter and Hurowitz, 1976; Liljequist, this symposium.)

In general our findings indicate that a state of CNS hyperexcitability persists long after the removal of alcohol. This covert CNS hyperexcitability may well be part of a larger "subacute post-withdrawal syndrome" which readily becomes reactivated by re-exposure to the addictive substance. One might speculate that this persisting subacute post-withdrawal syndrome may possibly contribute to an increased risk of returning to alcohol use in some as yet unspecified way. This is indeed suggested by two studies of craving in alcoholics. Recently Ludwig and Stark (1974) and Hore (1974) have indicated that the intensity and frequency of experiences of craving for alcohol in alcoholics are positively related to the severity of the withdrawal experienced during the most recent drinking episode and negatively related to the duration of abstinence.

The difference in neurophysiological responses to a small challenge dose of ethanol between our experimental and control animals indicates that facilitative reactivation of physical dependence is operative after a prolonged lapse of time. This implies that a once physically dependent organism, not exposed to alcohol for a period of time, can have that physical dependence reactivated with substantially smaller doses of ethanol than would be necessary in a naive animal. A somewhat related finding has been reported by Walker and Zornetzer (1974), but with a very different approach, using two successive alcoholization and withdrawal periods, with one week of abstinence between them. They demonstrated that EEG aberrations accompanying withdrawal are more severe following a second, although shorter alcoholization period than they are following an initial, longer alcoholization period.

We are presently investigating the relationship between the length and amount of exposure to alcohol, and the severity, persistence and permanence of CNS aberrations.

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