Tissue Responses to Addictive Drugs

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Brain Excitability Subsequent to Alcohol Withdrawal in Rats

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It has been postulated that a state of latent neural excitability develops during the alcoholization process, which is released upon termination of intake of the pharmacological agent (Seevers and Deneau, 1963; Kalent, et al., 1971; Mendelson, 1971). As a result of inherent methodological problems few studies have attempted to investigate withdrawal states in humans (Begleiter and Platz, 1972), making comprehensive animal research imperative.

Brain hyperexcitability in human alcoholics during alcoholization and withdrawal was examined using recovery functions of somatosensory evoked potentials by Begleiter et al. (1974). We found a progressive increase in brain excitability for each successive day of alcohol intake (during partial withdrawal), that reached a peak after one complete day of total abstinence. This finding was replicated in animals in a more recent study (Begleiter and Coltrera, 1975). Rats, implanted with recording electrodes at visual cortex and reticular formation received

intubated alcohol (or water) daily for 12 days. Visual evoked potentials obtained 24 hours after the last intubated dose indicated significant hyperexcitability in previously alcoholized rats. It was demonstrated that electrophysiological changes due to alcohol withdrawal far outlast overt behavioral aberrations.

It has been suggested by some investigators that central nervous system (CNS) disturbances persist far beyond the administration and removal of ethanol. In a study of human alcoholism, Mendelson, et al., (1966) found that when alcoholic and control subjects were subjected to an identical four-day period of alcoholization, only the alcoholics displayed withdrawal signs. In a similar study with animals, Branchey, et al., (1971) demonstrated that rats did not return to their pre-alcoholized state two-three weeks postwithdrawal; previously alcoholized animals exhibited severe withdrawal symptomatology when re-exposed to alcohol, unlike the naive matched-controls. Walker and Zornetzer (1974) studied both EEG and behavioral correlates of withdrawal from ethanol in mice. Withdrawal symptomatology was accompanied by abnormal EEG activity including spikes that often culminated in epileptic seizure discharges. Mice reintroduced and withdrawn from alcohol after an alcohol free interval, displayed more severe EEG disturbances than they had during their initial withdrawal episode. In another EEG experiment dealing with long-term CNS effects of alcohol, Gitlow et al. (1973) demonstrated that previously alcoholized animals displayed severe REM disturbances when re-exposed to alcohol after a six-month drug free period.

This chapter concerns itself with the attempt to investigate both short and long-term electrophysiological effects of chronic alcohol intake in rats.

MATERIALS AND METHODS

Twenty male hooded Long-Evans rats, with a mean weight of 358g were used in this experiment. They were housed individually in stainless steel cages, with ad lib access to food and water during the entire study.

Stereotaxic surgery was performed under Diabutal anesthesia (0.8cc/kg) for the purpose of recording visual evoked potentials (VEP's). Two monopolar teflon-coated stainless steel depth electrodes were implanted in the reticular formation and thalamus. Specific coordinates of the reticular formation 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 postventral nucleus of the

thalamus were: 3.0 mm posterior to bregma, 2.2 mm lateral to the midline (right), 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, 5.5 mm posterior to bregma and 4.0 mm lateral to the midline (left), and two similar screw electrodes placed bilaterally over the frontal cortex served as reference and ground. All leads were attached to a miniature connector and the assembly was fastened to the skull with acrylic cement. (Only data obtained from the visual cortex are presented in this chapter. Results from the other electrodes will be reported in a subsequent paper.)

The animals were allowed one to two weeks to recover from surgery, at which time they were placed in a sound-attenuated enclosure (IAC) and base line 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 a 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 - B Polygraph and fed into a PDP 11/40 computer for on-line signal averaging of a 500 msec epoch. Amplitude measures were obtained between 100 - 180 msec at N_2-P_2 for the visual cortex recordings.

Base line evoked potentials were obtained for each animal individually, following a habituation procedure. Throughout the experiment, each rat was tested on a carefully timed, staggered schedule such that only one rat was tested for base line, withdrawal, or challenge dose recordings per day.

Beginning on the morning following baseline determinations, 14 rats were intubated daily for 14 days, with an increasing progression of 20% (v/v) solution of 95% alcohol doses, (3 - 8g/kg) as follows: 3g/kg for the first two days; 4g/kg for the next two days; 5g/kg for two days, 6g/kg for two days, 7g/kg for the following four days, and 8g/kg for the remaining two days. Six control rats received an equivalent amount of water in the same fashion. VEP recordings were obtained beginning four 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=7) and half of the controls (N=3) received an alcohol challenge-dose (2 ml/kg 20% (v/v) of 95% ethyl alcohol) i.p., while the remaining animals received the same challenge dose after five weeks. (In this chapter we are only reporting the results obtained from the two-week challenge-dose group.) VEP's were recorded immediately preceding the

alcohol injection (base line) 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 postinjection.

DISCUSSION

Rats in both the experimental and control groups gained an average of 22g during the two-week intubation period, and weighed 380.26 and 380.83g, respectively at that time.

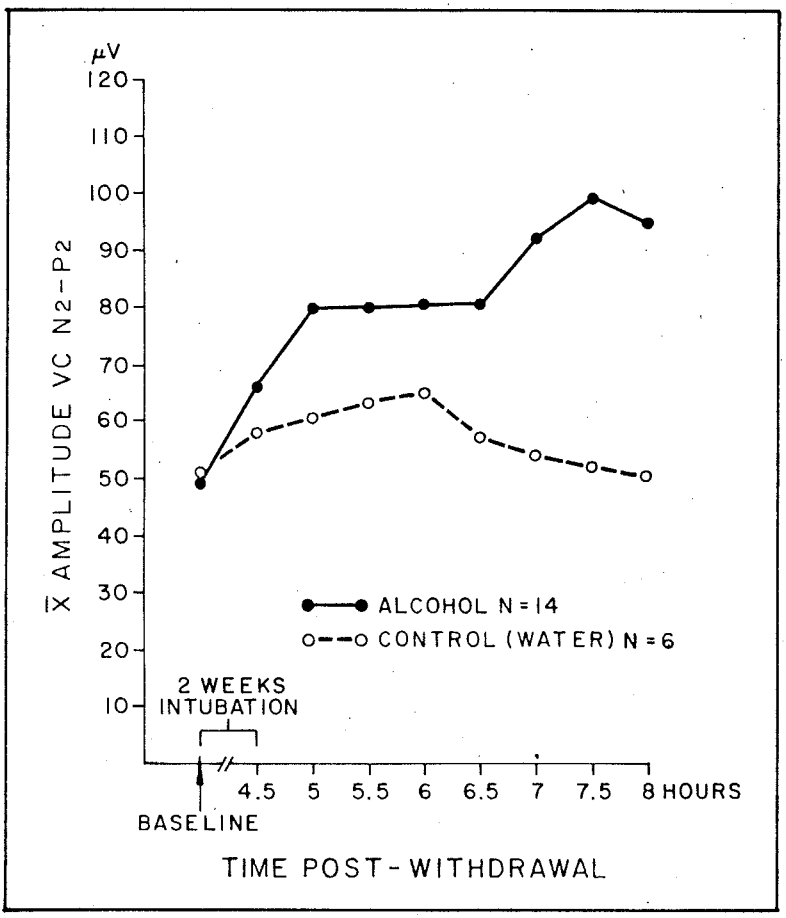
There were no significant differences in base line VEP's recorded before intubation between the experimental and control groups (see Figure 1). Beginning 6.5 hours after the last intubated dose until the end of the testing period, significant differences between the two groups were obtained as follows: $p < .05$ at 6.5 hours ($U=16$), $p < .02$ at 7 hours ($U=9$), $p < .002$ at 7.5 hours ($U=4$) and $p < .02$ at 8 hours ($U=15$). The maximum withdrawal effect was found at 7.5 hours post-withdrawal and was manifested by a marked increase in VEP amplitude.

Following two weeks of abstinence, base line VEP's recorded from the experimental and control groups did not differ significantly from each other. However, significant differences were obtained 40 minutes after each group received a challenge dose of alcohol ($U=1$, $p < .01$). The naive animals displayed a marked decrease in VEP amplitude at this time, while the experimental animals began to show increases which rose progressively over the course of the experimental session (see Figure 2). Significant differences in VEP amplitude were obtained between the two groups for the seven hours they were sampled (see Table 1).

CONCLUSIONS

The results indicate an increase in central nervous system (CNS) hyperexcitability during withdrawal in rats previously exposed to chronic alcohol intake. This neural hyperactivity is manifested by a marked increase in visual evoked potential (VEP) amplitude in those animals with a two-week exposure to alcohol. These findings are consistent with our previous results in animals (Begleiter and Coltrera, 1975), and with humans (Begleiter, et al., 1974).

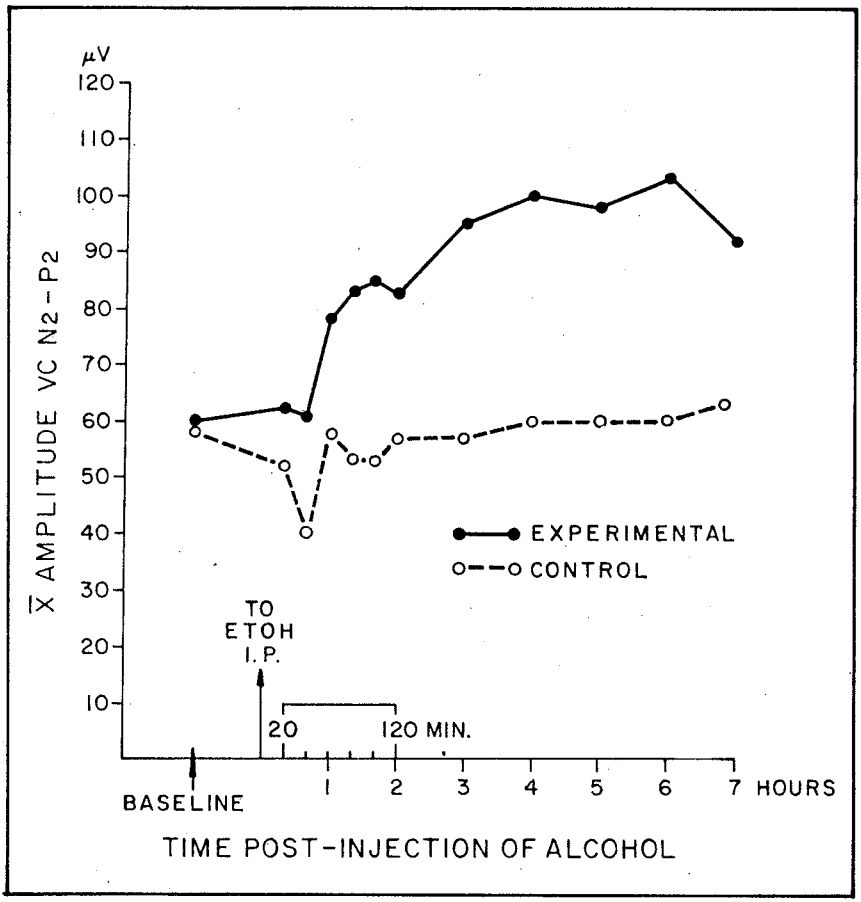
The time course of maximum CNS hyperexcitability obtained in the present study, namely 6.5 to 8 hours after the last alcohol intake, coincides with that reported by Hunter, et al. (1973) for rats, and Walker and Zornetzer (1974) for mice. In both studies, they monitored EEG in rodents that had received alcohol using a liquid diet technique,



1 Mean VEP amplitude N_2-P_2 recorded at visual cortex (VC) for 14 experimental and 6 control rats at base line (prior to treatment) and following two weeks daily intubation of either alcohol or water, respectively. Base line recordings are identical for the two groups, while VEP's sampled at half hour intervals from 4.5 - 8 hours postwithdrawal, indicate increasing differences between the two groups that are maximal 6.5 - 8 hours postwithdrawal, due to the progressively increasing amplitude in the experimental group.

and found that EEG withdrawal signs were most severe from six to ten hours postwithdrawal. In addition, their electrophysiological correlates of withdrawal followed a similar time course of development as we obtained in the present experiment.

The present investigation is not only concerned with the immediate



2 Mean VEP amplitude N_2-P_2 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). Base line determinations obtained on the same day prior to the challenge injection indicate no difference between the two groups. Naive animals (controls) with no previous exposure to alcohol respond with a sharp decrease in VEP amplitude, reaching asymptote 40 minutes postinjection, while previously alcoholized animals (experimental) display marked increases in amplitude peaking three-seven hours postinjection.

effects of chronic alcohol intake, but is also interested in possible long-term CNS abnormalities. While VEP recordings obtained following two weeks of abstinence from alcohol do not indicate any apparent differences between previously alcoholized animals and control rats, more subtle differences were found to exist between the two groups.

Table I.

Time after injection	U-value	P	Significance
base line	18	—	NS
20 minutes	4	0.072	NS
40 minutes	1	0.012	*
60 minutes (1 hour)	3	0.042	*
80 minutes	4	0.072	NS
100 minutes	3	0.042	*
120 minutes (2 hours)	4	0.072	NS
3 hours	0	0.006	*
4 hours	1	0.012	*
5 hours	1	0.012	*
6 hours	2	0.024	*
7 hours	0	0.006	*

*Significant at less than 0.05

Mann-Whitney U-tests performed on amplitude N_2-P_2 recorded at visual cortex between previously alcoholized and alcohol-naive rats, following a challenge dose of alcohol injected two weeks after treatment. No significant difference exists between the two groups at this time, prior to the challenge injection (base line). Significant differences between the two groups immediately after the alcohol injection (time 40 minutes) are due to a decrease in VEP amplitude in the control group, while later significant differences between the two groups (three-seven hours post-injection) are attributed to the increase in VEP amplitude in the experimental group.

The neurophysiological responses of the postaddict rats to a challenge-dose of alcohol are readily distinguishable from those of naive animals. Naive rats responded to a challenge-dose of alcohol with a typical immediate depression in VEP amplitude, while postaddict rats not only did not manifest this VEP decrease, but displayed instead a progressive increase in VEP amplitude, reaching levels of neural hyperexcitability as high as they had during the initial withdrawal period. This finding suggests that a state of CNS latent hyperexcitability underlies the alcohol addiction syndrome, and becomes reactivated when the organism renews contact with the addictive pharmacological agent. 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. In a previous experiment in our laboratory (Branchey, et al., 1971) we reported similar findings. The establishment of a state of physical dependence increased the incidence of withdrawal symptoms in a subsequent period of alcoholization. While no apparent withdrawal symptomatology was observed in naive animals, previously alcohol-dependent rats were prone to exhibit a severe withdrawal syndrome.

These findings, combined with those of the present investigation suggest the possibility that complex modifications in CNS responsivity occur in the addiction process. These changes can perhaps be considered as a form of long-term memory, an "addiction-memory," which lies dormant until it becomes reactivated by re-exposure to the addictive substance. In the present study, the mild dose of alcohol injected would normally not be sufficient to induce signs of hyperexcitability and, indeed, it did not in the naive animals. The full-blown excitability cycle that was elicited in the postaddict rats with this minimal dose of alcohol, suggests that a neurophysiological, adaptive change was already present in the postaddict rats, predisposing them to withdrawal symptoms. Evidence of a long-term biochemical change accompanying morphine addiction was reported by Sloan and Eisenman (1968). They demonstrated that postaddict rats secreted significantly less urinary norepinephrine than controls for a period of five months after morphine withdrawal, at which time adrenal norepinephrine levels were found to be elevated with considerable hypertrophy of the adrenal glands.

We are currently investigating the persistence of this neurophysiological dysfunction, its permanence, or possible irreversibility, by examining rats that are re-exposed to alcohol following various intervals of abstinence. In addition, the relationship between the length of exposure to alcohol, and the severity and persistence of neurophysiological aberrations are being presently examined by studying CNS hyperexcitability in rats subjected to chronic alcohol administration for different lengths of time. The mechanisms involved, and the manner in which this reactivation of CNS hyperexcitability is accomplished, remains to be investigated.

SUMMARY

In conclusion, our present data indicate that the effects of ethanol on an organism far outlast the administration and removal of the pharmacological agent; organisms appear to undergo adaptive modifications that directly affect CNS responsivity, and may be considered as

a form of memory ("addiction-memory"). The nature, time course of development, and duration of this altered neurophysiological responsivity ("addiction-memory") remain to be examined during the induction, withdrawal, and postwithdrawal phases of alcohol addiction.

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