PROTRACTED BRAIN DYSFUNCTION AFTER ALCOHOL WITHDRAWAL IN MONKEYS

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INTRODUCTION

The alcohol withdrawal syndrome includes a broad spectrum of severity. The milder stages of the syndrome include such symptoms as slight tremor, malaise and general irritability, and the more severe stages of the syndrome are characterized by delirium tremors. While the nature of the underlying mechanisms is not established, the evidence presently available suggests that there may well be different mechanisms for different manifestations associated with withdrawal.

A basic general model associated with drugs producing tolerance and physical dependence, such as alcohol, barbiturates and opiates is that some functions changed by the presence of the drug react to the elimination of that particular drug by a drastic shift in the opposite direction. This shift in the opposite direction is often accompanied by an overshoot or rebound before attaining a relatively normal level. Sharpless (1964) and Jaffe and Sharpless (1968) had suggested a "denervation supersensitivity" hypothesis to account for these events. Other cogent hypotheses have been proposed by Mendelson (1971) and Kalant (1973). An early and particularly clear demonstration of this phenomenon was provided by the work of McQuarrie and Fingl (1958). They demonstrated that the increased convulsive threshold during alcohol intake in mice was invariably followed by a sharp rebound with lowered convulsive threshold during withdrawal.

It has been suggested that this rebound phenomenon is the manifestation of a state of latent hyperexcitability in the central nervous system. It is generally accepted that the clinically observable behavioral signs of withdrawal reflect this neuronal hyperexcitability. A number of electroencephalographic investigations have been
conducted in human subjects in an attempt to examine this hyperexcitability. This body of literature has been reviewed by Begleiter and Platz (1972).

In 1974, the first direct electrophysiological evidence of hyperexcitability in alcoholics during withdrawal was reported by Begleiter, Porjesz and Yerke-Grubstein (1974). These investigators used the recovery function of somatosensory evoked potentials to examine brain excitability in alcoholic patients. The recovery function was determined by administering pairs of stimuli separated by varying intervals. The ratio of voltages of the second neuroelectric response over the first indicated the extent to which responsiveness was recovered after a given interval of time. Recovery functions obtained 10 hours after alcohol discontinuation indicated a significant increase in neuronal excitability even after only one day of drinking. The results indicated a progressive increase of brain hyperexcitability starting a few hours after the intoxication period and reaching asymptote with the first day of total withdrawal from alcohol.

Subsequently we have conducted a series of experiments to investigate the persistence of this neural hyperexcitability following cessation of alcohol intake. We reported (Begleiter and Coltrera, 1975) that neural hyperexcitability was observed in rats 24 hours after their last dose of ethanol. Porjesz, Begleiter and Horowitz (1976) reported that the electrophysiological responses of rats previously exposed to alcohol were significantly different from those recorded from naive animals.

In a subsequent experiment we (Begleiter and Porjesz, 1977) investigated the persistence of these neuroelectric changes. Rats were implanted with electrodes in various brain sites in order to record baseline evoked potentials prior to the introduction of ethanol. Animals were intubated daily with a progression of increasing quantities of 20% (V/V) alcohol (1-8g/kg), while other animals received an equivalent amount of water administered in the same fashion. Beginning 48 hours after the last dose of intubated alcohol, evoked potential recordings were taken every half-hour up to 8 hours and from 24 to 72 hours post withdrawal. The findings indicated that all experimental animals manifested the most significant neural hyperexcitability between 7 and 8 hours after alcohol withdrawal. It should be noted that 24 hours after alcohol withdrawal their brain hyperexcitability had not yet returned to baseline.

Following two weeks of total abstinence, half of the experimental group, and half of the control group received an alcohol challenge dose (2g/kg. i.p.), while the remaining animals received the same challenge dose after five weeks. Significant hyperexcitability was observed in the two-week challenge dose animals that had been previously subjected to alcohol intake. No such effect was observed in the control animals. While we noticed some evidence of hyperexcit-
ability after five weeks of abstinence from alcohol in the experimental group, this effect did not reach statistical significance. These data indicate that the neurophysiological responses of post-addict rats to challenge doses of alcohol are readily distinguishable from those of naive animals. This effect reflects the presence of a protracted neuronal hyperexcitability which is only present at specific brain sites.

In order to test the generality of our findings and to investigate the brain specificity of the aforementioned results further, we conducted similar experiments in the monkey.

METHODS

Four Bonnet monkeys (Macaca Radiata) with a mean weight of 4.1 kg were used in the experiment. Stereotaxic surgery was performed while the animals were anesthetized with acepromazine meleate (3.0 mg/kg IM) and sodium pentobarbital (20.0 mg/kg IV) under sterile conditions. All animals were implanted with monopolar teflon-coated stainless steel depth electrodes at the following sites: lateral geniculate body (LGB), supracryptic nuclei (SO), pulvinar (P), dorsal hippocampus (HIPP), ascending mesencephalic reticular formation (MRF), visual cortex (VC), frontal association cortex (FC), and parietal association cortex (PC). Two stainless steel 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 4 weeks to recover from surgery during which time they were adapted to a monkey chair (BRS/LWE), which was located inside a sound-attenuated RF shielded room (IAC enclosure). Baseline visual evoked potentials (VEP's) were recorded to a total of 50 repetitive (1/2 sec) foveally presented light flashes, generated by a Grass PS-2 photic stimulator (intensity=8) located 24 inches from the animal's eyes. During all recording sessions the animal's head was restrained in order to minimize head movements.

Visual evoked potentials (VEP) were amplified by a Grass Model-78B Polygraph and fed into a PDP 11-40 computer for on-line signal averaging of a 500 msec epoch. All data were digitized and stored on computer disks or magnetic tape for subsequent analysis. Amplitude and latency measures were obtained for all averaged evoked potentials recorded at all electrodes. Only the major early and late components of each evoked potential were measured.

Immediately following evoked potential baseline recordings, the intubation procedures were begun. The experimental monkeys were intubated with alcohol (5.0g/kg of 25% w/v solution) once a day for thirty consecutive days, while the two control monkeys were intubated with iso-caloric sucrose following an otherwise identical regimen. Both groups were also intubated with 2.0 ml of poly-vi-sol multiple
vitamin drops every other day through the 30 day period.

Twenty minutes prior to the last intubation (Day 30) visual evoked potentials (VEP's) were recorded following the identical procedure described for the baseline recording sessions. The experimental animals were then intubated with alcohol and the control animals with iso-caloric sucrose. VEP's were then recorded every 20 minutes for the first two hours and every hour thereafter for 24 hours. During this time, blood (1.0 ml) was collected from a stab wound in the foot and analyzed for alcohol concentration. It was collected every 20 minutes for the first two hours, every hour for the next 6 hours, and every other hour thereafter until the Blood Alcohol Concentration (BAC) reached zero. The blood samples were prepared with Calbiochem Ethyl Alcohol Reagents and analyzed by enzymatic procedures.

Following the 24 hour VEP procedures, the monkeys were returned to their home cages. During the next 37 days, the animals were maintained under standard laboratory conditions with food and water ad lib. After this 37 day period, the monkeys were placed back in the restraint chair and VEP's were recorded once again. Twenty minutes later, all monkeys were intubated with a 2.0g/kg dose of alcohol (25% w/v solution) and VEP's and BAC were obtained for a full 24 hour period as described above.

In this paper, only the visual evoked potential amplitude data collected from all monkeys in response to the challenge dose of alcohol following the 37 day recovery period are presented. For the sake of simplicity, these amplitude data are presented in terms of post-challenge percent change from baseline.

RESULTS

Analysis of variance with repeated measurement (Winer, 1962) were performed on the VEP amplitude percentage change scores obtained from each brain electrode: lateral geniculate body, supraoptic nuclei, pulvinar, dorsal hippocampus, ascending mesencephalic reticular formation, visual cortex, frontal association cortex and parietal association cortex.

Figure 1 demonstrates the amplitude changes recorded from the supraoptic nuclei of the control and experimental animals during the 24 hour period following the challenge dose of ethanol. The difference between the control and experimental animals was not statistically significant. The differences between the evoked potential amplitudes recorded from control and experimental monkeys at lateral geniculate body (Figure 2), pulvinar (Figure 3) and visual cortex (Figure 4) were not statistically significant either. In contrast, the amplitudes of VEP's recorded from control and experimental animals subsequent to a challenge dose of alcohol were significantly
Figure 1.

Mean percent change in VEP amplitude from pretreatment baseline (B) in previously alcoholized monkeys (----) and control monkeys (-----) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at supraoptic nuclei prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 2.

Mean percent change in VEP amplitude from pretreatment baseline (--) in previously alcoholized monkeys (----) and control monkeys (-----) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at lateral geniculate body prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 3.

Mean percent change in VEP amplitude from pretreatment baseline (B) in previously alcoholized monkeys (----) and control monkeys (-----) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at pulvinar prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 4.

Mean percent change in VEP amplitude from pretreatment baseline (8) in previously alcoholized monkeys (- - -) and control monkeys (---) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at visual cortex prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 5.

Mean percent change in VEP amplitude from pretreatment baseline (B) in previously alcoholized monkeys (-----) and control monkeys (-----) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at mesencephalic reticular formation prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 6.
Mean percent change in VEP amplitude from pretreatment baseline (B) in previously alcoholized monkeys (-----) and control monkeys (----) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at hippocampus prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 7.

Mean percent change in VEP amplitude from pretreatment baseline (B) in previously alcoholized monkeys (——) and control monkeys (——) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at frontal cortex prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 8.

Mean percent change in VEP amplitude from pretreatment baseline (B) in previously alcoholized monkeys (----) and control monkeys (-----) following 37 days of abstinence. Percentage changes in VEP amplitude recorded at association cortex prior to an alcohol challenge dose (time 0), every 20 minutes for the first 2 hours post-injection (2g/kg ETOH i.p.), and at hourly time intervals thereafter are indicated.
Figure 9.

Mean blood alcohol levels (BAL's) in previously alcoholized monkeys (---) and control monkeys (-----).

The top trace indicates BAL's at pretreatment baseline (B) and on the last day of alcohol intubation following 30 days of alcohol exposure for the experimental group. BAL's following the last intubation (5g/kg) are indicated every 20 minutes for the first 2 hours and every hour thereafter for 24 hours.

The bottom trace indicates BAL's at pretreatment baseline (B) for both the previously alcoholized monkeys and the control monkeys following 37 days of abstinence after receiving an alcohol challenge dose (2g/kg i.p.). BAL readings are indicated at 20 minute intervals for the first 2 hours and every hour thereafter until the BAL has returned to 0 (0-11 hours, post-injection).
different at the following sites: mesencephalic reticular formation (Figure 5), hippocampus (Figure 6), frontal association cortex (Figure 7) and parietal association cortex (Figure 8).

It should be noted that the maximum neural hyperexcitability occurred at different times for the various recording sites. In the experimental animals, maximum hyperexcitability was reached between 7 and 10 hours after the challenge dose at hippocampus, frontal and posterior association cortices; at mesencephalic reticular formation, the neural hyperexcitability reached asymptote between 8 and 16 hours after the challenge dose of alcohol. Figures 1 to 8 also illustrate that the challenge dose of alcohol induced a significantly greater percent decrease in evoked potential amplitudes recorded from the control animals than those obtained from the experimental animals at all brain sites. Maximum amplitude depression occurs between 1-3 hours post-challenge, at all electrodes, the time range corresponding to peak BAC levels (Figure 9).

It should be noted that the mean weight for the control and experimental animals did not differ at the beginning and at the end of the experiment. The blood alcohol levels obtained from the experimental animals on the last day of intubation are illustrated in the top of Figure 9. The bottom graph of Figure 9 demonstrates the blood alcohol levels obtained from all animals for 24 hours following the injection of the challenge dose of alcohol (2g/kg). As can be seen, the blood alcohol curves for the experimental and control animals are quite similar.

**DISCUSSION**

Our results indicate that chronic alcohol intake results in central nervous system changes which persist long after the withdrawal from alcohol. These long lasting CNS changes reflect a state of latent neural hyperexcitability which becomes reactivated subsequent to reexposure to alcohol.

The present findings are quite consistent with previous data from our laboratory (Begleiter, Porjesz and Yerre, 1974; Begleiter and Coltrera, 1975; Porjesz, Begleiter and Hurowitz, 1976; Begleiter and Porjesz, 1977; Begleiter and Porjesz, 1978; Begleiter and Porjesz, 1979) which indicate that a state of CNS hyperexcitability is present long after the removal of alcohol. These persisting levels of anomalous hyperexcitability have been observed in animals by other investigators (Branchey, Rauscher and Kissin, 1971; Walker and Zornetzer, 1974; Freund and Walker, 1971; Gitlow, Bentkover, Dziedzic and Khazan, 1973).

It should be noted that the existence of a protracted subacute withdrawal syndrome has been well-documented in the clinical literature. This persistence of withdrawal symptoms has been studied more
extensively in heroin abuse than in alcohol abuse. Martin and Jasinski (1969) found that narcotic addicts continued to show evidence of psychological aberrations (miosis, hyperthermia, tachycardia, increased catecholamine excretion) for periods lasting at least six months after withdrawal. Martin et al., (1968) reported decreased respiratory response to CO₂ levels while Himmelsbach (1942) reported an increased cold pressor response. In drug addicts, insomnia persists for at least six weeks following withdrawal, while slow wave sleep (SWS) is substantially suppressed for approximately 13 weeks after withdrawal (Kay, 1975).

The persistence of withdrawal symptoms has also been observed in alcoholics. Autonomic nervous system aberrations lasting at least three weeks have been reported by Kissin et al., (1959). Abstinent patients displayed respiratory irregularities and poor inhibitory control of fine motor movements. Cold pressor response was reduced and overactivity of parasym pathetic functions was noted. These long lasting deficits also appear in the sleep characteristics of abstinent alcoholics (Gross et al., 1973; Johnson, 1971; Wagman and Allen, 1975).

Our present findings indicate that 37 days after the last dose of alcohol, a state of neural hyperexcitability can be reactivated when the organism renews contact with the addictive pharmacological agent. Similar findings were reported by Walker and Zornetzer (1974). They used two successive alcoholization and withdrawal periods with one week of abstinence intervening between them. They reported that EEG aberrations accompanying withdrawal are more severe after a second, although shorter alcoholization period than they are following an initial albeit longer alcoholization period. In a similar experiment, 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.

The reactivation of withdrawal symptoms after a short period of exposure to alcohol has also been observed in humans by Mendelson, Stein and McGuire (1966). These authors 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 symptoms, while none of the controls did. The authors interpreted their results as indicating that alcoholic patients have a long lasting predisposition to develop withdrawal symptoms.

The persistence of this predisposition to neural hyperexcitabil-
ity is supported by our present results. The electrophysiological responses of the experimental animals are significantly different from those of the control animals after the administration of a challenge dose of alcohol. Our results indicate that these changes in neural hyperexcitability are reflected by an increase in amplitude of the evoked potentials. The neural specificity of this latent hyperexcitability is manifested by the increased amplitude of evoked potentials recorded at selected neural sites. Neural hyperexcitability was obtained at reticular formation, hippocampus, frontal cortex and parietal cortex, whereas the supraoptic nuclei, lateral geniculate body, pulvinar and visual cortex did not manifest a significant increase in neuroexcitability. While our results indicate that hyperexcitability may be specific to certain brain regions, great caution is required in the interpretation. It is critical to understand that increases in the voltage of the evoked brain potentials cannot at present differentiate between excitatory or inhibitory cellular activity. Therefore, it must be understood that this neurophysiological finding is only specific to the number of brain sites sampled in our present experiment. The observed neural hyperexcitability may also be specific to the sensory evoked potential under study: namely, the visual evoked potential. Finally the observed hyperexcitability may not reflect an increase in neuroexcitability at the recording electrode but may indeed be indicative of changes in neural excitability at some distant generator or proximal neural aggregate. In spite of the difficulties in the neurophysiological interpretation of these findings, our data do not suggest that hyperexcitability is all pervasive throughout the brain. Our results strongly support the notion that CNS hyperexcitability associated with the withdrawal syndrome is specific to certain brain sites and is reflected behaviorally by the manifestation of specific clinical symptoms.

We (Begleiter and Porjesz, 1979) have previously suggested that this latent neural hyperexcitability may reflect the presence of a "subacute post-withdrawal syndrome" which readily becomes reactivated by reexposure to the substance of abuse. We have speculated that this protracted subacute withdrawal syndrome may possibly contribute to an increased risk of returning to alcohol use in some as yet unspecified way. This suggestion is supported by a recent study from our laboratory (DeNoble, V.D. and Begleiter, H., 1978). We observed that monkeys previously exposed to alcohol, self-administered significantly more alcohol during the first two alcohol test days than naive animals. Subsequently the rate of self administration for both groups was quite similar. It is important to note that the prior exposure to alcohol in the experimental animals occurred four months prior to the self-administration period.

In brief, our present data indicate that chronic alcohol abuse results in CNS changes which appear to be specific and persisting for a relatively long period of time. These CNS aberrations may
represent a subacute withdrawal syndrome not readily observable but of great potential clinical significance.

REFERENCES


