

## **Evoked Potential Changes During Ethanol Withdrawal in Rats\***

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### ABSTRACT

We have recently observed an increase in central nervous system excitability during intoxication and alcohol withdrawal in alcoholics. Our present results demonstrate a similar phenomenon in rats. The hyperexcitability caused by ethanol withdrawal is still present subsequent to the overt behavioral symptoms of withdrawal.

There is extensive literature concerning the behavioral signs of withdrawal from alcohol [1-3]. It has often been hypothesized that the manifestations of withdrawal symptoms subsequent to removal from alcohol may reflect a state of latent neural hyperexcitability [4]. However, the investigation of central nervous system aberrations in humans during withdrawal has been hampered by numerous methodological difficulties [5].

Recently, Begleiter *et al.* [6] used the recovery cycle of somatosensory evoked potentials to study changes in brain excitability in human alcoholics

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during intoxication and withdrawal. A recovery function was always determined in the morning, 10 hours after the last drink, during three days of baseline, four days of alcoholization, and four days of withdrawal from alcohol. Our findings indicated a progressive increase of brain excitability starting with the intoxication period and reaching asymptote with the first day of total alcohol withdrawal.

Because of various methodological limitations in pursuing our investigation of central nervous system changes during alcohol withdrawal in humans, we undertook to study such changes in animals. The present experiment is, first, an attempt to replicate and extend our past findings with humans to animals and, second, to investigate the possible central nervous system abnormalities which might outlast the behavioral symptoms of alcohol withdrawal.

## METHODS

Ten Long Evans rats, weighing 250-300 g, were implanted under pentobarbital anesthesia with stainless-steel screws placed bilaterally over the frontal sinus which were used as references. One stainless-steel screw was placed on the right side of the visual cortex 2-3 mm anterior to Lambda, and 3-4 mm lateral to midline. A monopolar stainless-steel electrode, covered with Teflon and exposed at the tip for 0.05 mm was implanted in the ascending reticular formation, 6 mm posterior to Bregma, and 2.5 mm lateral to midline. The leads were attached to a miniature connector, and the assembly was fastened to the skull with acrylic cement. All animals were allowed one week to recover from surgery. Subsequent to surgery, the animals were randomly assigned to the control or experimental group.

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 a PS-2 Grass photostimulator located outside the one-way vision mirror of the sound-attenuated enclosure. The stimulus was delivered regularly at a rate of 1/2 sec, for a total of 64 stimuli.

Evoked responses were amplified by a Beckman Polygraph and summed by a Mnemotron computer of average transients (CAT 1000) set to analyze

electrocortical activity for 500 msec after each flash. The summed responses were plotted by an *X-Y* plotter. One week subsequent to surgery the animals were habituated to the recording environment and a baseline evoked potential was determined for all animals. After the baseline determination, the experimental animals were intubated daily with specific ethanol solutions as follows: 4 g/kg for the first 3 days, 5 g/kg for the next 3 days, 6 g/kg for the next 3 days, 7 g/kg for the following 2 days, and 8 g/kg for the final day before total removal of ethanol. Throughout the entire experiment, animals were given food and water ad libitum.

After 12 days of intubation, alcohol was withdrawn and evoked potentials were recorded 24 hours after the last intubation. We chose a period of 24 hours because it has been reported that rodents [7-9] do not appear to manifest overt behavioral signs of withdrawal past a 10-12 hour period.

Amplitude determinations were made for the evoked potentials obtained at the reticular formation and the visual cortex in accordance with criteria reported previously [10]. Only the most marked and stable voltage deflections were used to make the peak to trough measurements. In the case of the visual cortex potentials, the  $N_1-P_2$  component occurring at approximately 50 msec and the  $N_2-P_2$  components occurring between 100 and 180 msec were measured. With regard to the reticular formation only the  $P_1-P_1$  component occurring at approximately 35 msec was taken.

## RESULTS

The results indicate no significant difference between experimental and control animals for baseline evoked potentials. The evoked potential differences recorded during withdrawal were significant at the visual cortex as follows:  $N_1-P_2$  significant at  $p < 0.02$ ;  $N_2-P_2$  significant at  $p < 0.05$ . The differences in evoked potentials recorded at the reticular formation of experimental versus control animals were not significant (Table 1).

## DISCUSSION

Our findings indicate that withdrawal from ethanol subsequent to chronic intake results in increased evoked potentials indicative of central

Table 1. Amplitude Measurements (microvolts) Obtained from Visual Cortex and Reticular Formation of Experimental Animals and Control Animals

Animals	$R_1$	$R_2$	$R_3$	$R_4$	$R_5$
Visual Cortex					
$P_1N_1$					
Exp. Rats	84.5	56	142	89.5	86.5
Control Rats	86	38.5	56	70	67
$N_2P_2$					
Exp. Rats	151	172	126	105	155.5
Control Rats	96.5	88.5	97	143	138
Reticular Formation					
$N_1P_1$					
Exp. Rats	38	40	17.5	10.5	50
Control Rats	14.5	37	36	22	30
$P_1N_2$					
Exp. Rats	41	77	55.5	49	105.5
Control Rats	56	68	19	33	63.5

nervous system hyperexcitability. Our present animal data are quite consistent with our previous findings obtained in alcoholics [6].

Recently, electroencephalographic changes recorded during alcohol withdrawal have been reported.

Walker and Zornetzer [9] used the liquid diet technique to induce withdrawal symptoms in mice after 6 days of continuous consumption. The mice were observed for a period of 6-8 hours subsequent to withdrawal. During that time isolated spike events were recorded from several brain structures, often culminating in sustained epileptic seizures. Hunter *et al.* [7] administered a liquid diet to rats for a period of 16 days. The alcohol diet was then withdrawn and the rats were monitored for 8-10 hours post-withdrawal. They noted that the withdrawal symptoms were most severe 6-10 hours post-withdrawal. EEG epileptiform activity was observed including sustained paroxysmal activity.

It should be noted that the above behavioral and electroencephalographic abnormalities during post-withdrawal were all observed within 6-10 hours after the removal of alcohol. Our present findings indicate that CNS changes caused by alcohol withdrawal last much beyond the 10-hour period reported above. Our results indicate that 24 hours post-withdrawal, when behavioral abnormalities are no longer readily observable, the evoked

potentials of experimental animals are significantly different from those of control animals. Our data indicate that the evoked potential changes brought about by alcohol withdrawal are found at the visual cortex and not at the reticular formation.

It is quite unlikely that the neural aberrations found during alcohol withdrawal are generated in the visual cortex and not present in the reticular formation. However, it is quite possible that the greater and more enduring effects of alcohol withdrawal are exerted on cortical rather than on subcortical structures.

It has been suggested by Hunter *et al.* [7] that the cortex does not play a primary role in the genesis of behavioral hyperexcitability normally observed during alcohol withdrawal. Nevertheless, disturbances in cortical function last for a minimum of 24 hours and may be quite long lasting. We are presently investigating the duration of persistent central nervous system changes subsequent to chronic alcohol intake.

The findings on the long-term effects of alcohol may be of special clinical significance in the search for possible treatment modality and in determining the length of hospitalization necessary.

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