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Effects of Ethanol Inhalation on EEG in Rats

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GHOSH, T K , R L COPELAND, JR AND S N. PRADHAN *Effects of ethanol inhalation on EEG in rats* PHARMACOL BIOCHEM BEHAV **38**(2) 293–297, 1991 —Effects of ethanol on duration of stages of sleep-wake cycle and EEG power spectra were measured during a 2-h exposure in a dynamic inhalational chamber in rats Rats were exposed to one of four graded concentrations (approx 100, 400, 800 and 1600 ppm) of ethanol on different days Ethanol was found to increase the duration of waking (W) with a decrease in duration of rapid eye movement (REM) sleep at 100 and 400 ppm No effect was observed at 800 and 1600 ppm on the stages of sleep-wake cycle or at 100–1600 ppm on EEG power spectra from the somatosensory or visual cortices Results indicate that ethanol administered by inhalation could produce arousal action at low doses, but did not have any effect on EEG power spectrum at the concentrations used

Ethanol EEG Power spectra Sleep-wake cycle

EFFECTS of ethanol on EEG have been studied in humans as well as in several species of experimental animals. In moderate to high doses (2–4 mg/kg) ethanol produces a shift toward lower dominant alpha frequencies, as drowsiness sets in [(10,16); see (2)]. Lower (<2 mg/kg) intoxicating doses show less consistent effects varying from generalized low-voltage, high-frequency activity to no EEG effects to generalized high-voltage, low-frequency activity (2); such conflicting data may be due to species difference, route of administration or method of EEG analysis. In these experiments ethanol has been administered to subjects by various routes and procedures, such as feeding through stomach tube, mixing with liquid diet, IV injection, etc.

Since ethanol is used as an industrial solvent, workers are exposed to its vapor in the work place. Although ethanol, one of the most abused chemicals, is almost always abused by oral consumption, it has also been reported to be self-administered by inhalation to achieve euphoria (1). Ethanol has been used by inhalation (along with use of pyrazole, an alcohol dehydrogenase inhibitor) to produce tolerance and physical dependence in mice (6–8). Ethanol administered by inhalation has been shown to cause a decrease in response rate in fixed-ratio and fixed-interval reinforced behavior in mice, but at high concentrations (11,12). In behavioral studies from our laboratory, inhaled ethanol also caused a decrease in reinforcement rate in fixed-ratio liquid-reinforced behavior as well as self-stimulation behavior in rats (5). In the present experiment, effects of ethanol during its inhalation were studied on the sleep-wake cycle and electroencephalogram (EEG) power spectrum in rats

METHOD

Animals

Male rats (F344, Charles River Breeding Lab) having initial body weights of 180–210 g were used. They were housed individually in stainless steel cages. Animals were kept in artificial light-dark cycle of 12/12-h (light 7 a.m to 7 p m.) and the mean ± S.E. of the temperature and relative humidity of the animal room were 24 ± 1°C and 55 ± 5%, respectively

EEG and Power Spectrum Analysis

Surgical procedure. Implantation of electrodes, one on each side over the somatosensory as well as the visual cortices for EEG recording and one into the neck muscle for EMG recording, was according to the procedure of Ghosh et al. (4).

Polygraphic recordings and classification of sleep-waking stages. Two days after the surgery, each rat was placed in the inhalational chamber (see later) for 3 days of habituation, while EEG and EMG were recorded via a Grass Model 7 Polygraph for 6 h daily (9 30 a.m. to 3·30 p.m.). EEG frequencies from 0.3 to 30 Hz and EMG frequencies from 5 to 75 Hz were allowed to pass through the filter. Further details of the polygraphic recordings were the same as in Ghosh et al. (4). When the data from the last two consecutive days were consistent, rats were exposed to ethanol for 2 h (12·30 p.m. to 2 30 p.m.). One-hour recording preceding the exposure was considered as the preexposure con-

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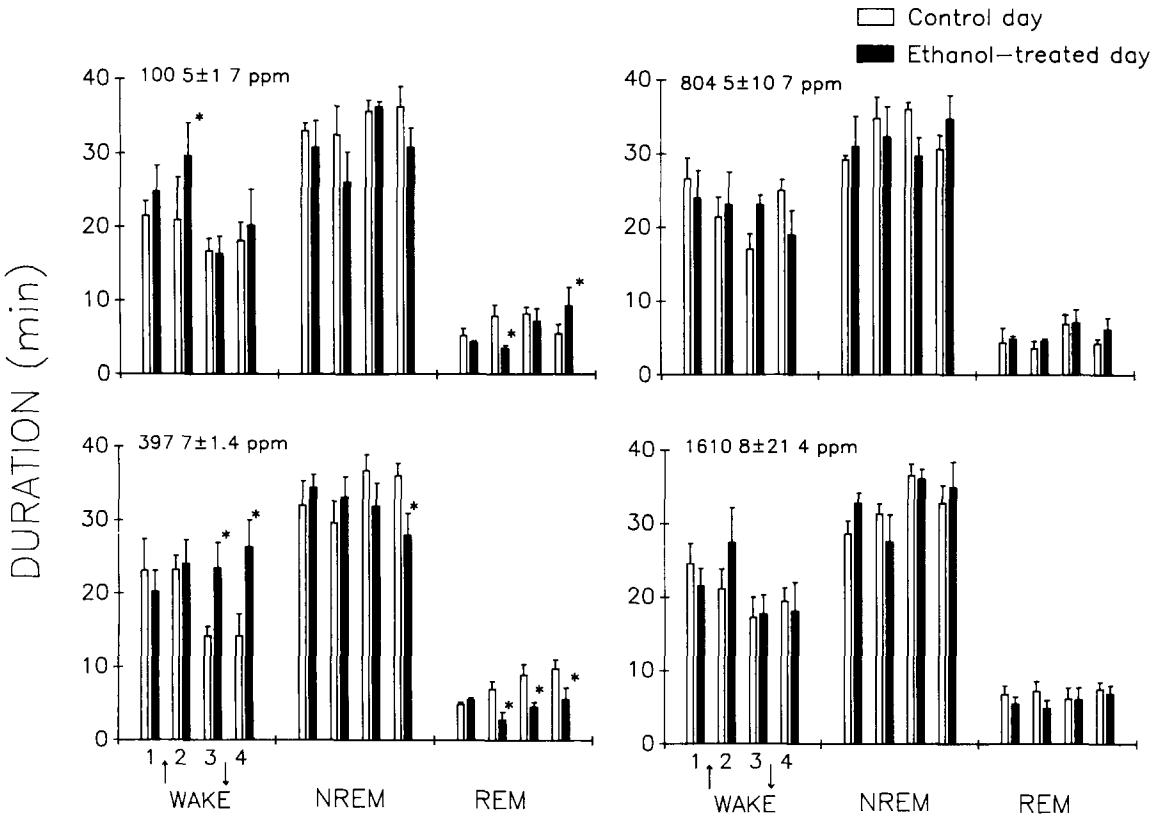

FIG 1 Effects of ethanol inhalation at four different concentrations on the hourly duration of awake, nonrapid eye movement (NREM) sleep and rapid eye movement (REM) sleep during two-hour exposure in 4-h session. 1 preexposure hour, 2 and 3 h-1 and h-2 of ethanol exposure (indicated by arrows), 4 postexposure hour Durations of each time period are compared between previous day control (control day) and ethanol-treated day *$p<0$ 05

trol, and the postexposure effect was recorded during the last hour. Data from the day preceding the exposure were considered as the previous day control.

Polygraphic recordings of the rat were grouped into three stages, as already described (4): 1) waking (W) characterized by the low-amplitude EEG from both the somatosensory and visual cortices and the high-amplitude EMG, 2) nonrapid eye movement (NREM) sleep characterized by the high-amplitude irregular EEG from both the cortices and low-amplitude EMG; 3) rapid eye movement (REM) or paradoxical sleep (PS) characterized by the low-amplitude EEG from the somatosensory cortex, continuous θ waves in the visual cortex and the low-amplitude EMG. The θ activity originating from the electrode over the visual cortex during REM sleep was confirmed with spectral analysis.

Power spectrum analysis. EEG and EMG activities were also recorded on a FM magnetic tape recorder (A.R. Vetter Co., Model C4) Power spectral analysis of EEG was performed off-line using a Nicolet MED-80 minicomputer system which uses Fast Fourier Transformation for computation [for further details see Ghosh et al. (4)]. EEG power spectra were derived from 10-s samples of EEG that were digitized at a sampling rate of 50/s and power spectral densities were estimated from 0 to 25 Hz and plotted on a X-Y plotter. The digital values of power spectra of the four major frequency bands, δ(0–4 Hz), θ(4–7 Hz), α(8–13 Hz) and β(14–20 Hz) from six to twelve 10-s EEG samples (for details see the Statistical Analysis section) during each of W, NREM and REM sleep stages were obtained from the printout.

Exposure to Ethanol

Rats were exposed to ethanol (U.S. Industrials Co.) in a dynamic inhalational behavioral chamber devised by Pradhan and Copeland (15). The chamber consisted of an inverted cylindrical glass chromatography jar which covered a circular grid floor. The chamber was infused with a flow of air derived from house air supply. The house air was filtered, mixed with ethanol vapor in an evaporating flask and passed through a water-cooled condenser to lower the temperature of the mixture before entering into the exposure chamber

Rats were exposed to one of the four concentrations of ethanol estimated to be 100, 400, 800 and 1600 ppm for 2 h in an ascending order on different days. The same rat was not exposed to the next concentration for at least 15 days to prevent the effect of repeated ethanol exposure

The concentration of ethanol in this chamber was monitored by collecting gas samples in a sampling bulb from inside the chamber at 15-min intervals, and then injecting 1 ml of the sample into a Shimadzu dual-column gas chromatograph (GC) equipped with flame ionization detectors (Model GC Mini 2) The ethanol concentration was measured with the help of a digital integrator connected to the GC. The average concentration of ethanol in samples collected during a daily session with a rat was expressed

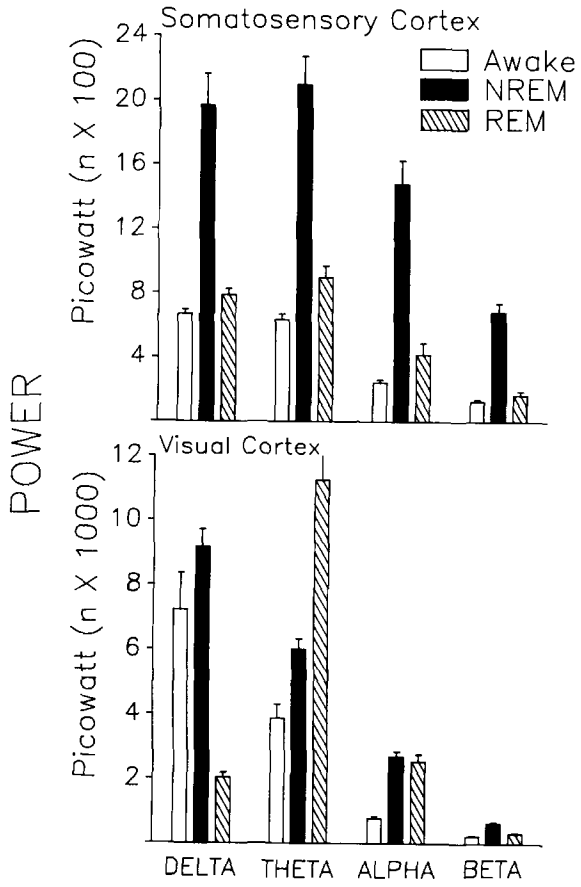

FIG 2 Power spectra of EEG recorded from two cerebral cortices during different stages of sleep-wake cycle in the control condition

as the grand mean ± S.E.

The grand mean of ethanol concentration for each rat at a particular estimated concentration level was pooled together to calculate the overall mean and SE, as shown in each experimental category. There were at least 4 rats in each of the two experimental categories sleep-wake cycle and power spectrum studies. In each category same rats were used at each ethanol concentration in graded sequence at stated intervals.

Statistical Analysis

Sleep-wake stage analysis. The behavioral stage duration variables (wake, REM sleep, NREM sleep) were analyzed for rats to identify significant changes from the previous day control in any hour. For each animal, the difference between the exposure day value and the control day value, during each hour, was obtained. These differences were analyzed with a cell means model in the general linear models procedures for two-way analysis of variance (ANOVA) the main factors being dose level and time in chamber followed by the Dunnett's post hoc test, so that the exposure/control comparison is modeled directly, yielding single degree-of-freedom tests of significance ($p<0.05$), for each exposure by time cell.

Power spectrum analysis The spectral power variables were also analyzed to identify significant changes from control in any hour. The control value for each animal was determined for each stage and exposure level by taking the mean of twelve 10-second EEG samples during the preexposure control period (one hour before exposure) and twelve 10-second EEG samples during various times on the previous day. Power spectra of six 10-seconds of each stage during the last halves of the first and second hours of the exposure period, and the last half of the first postexposure hour, were calculated for each of the spectral power variables, at each exposure level and stage. Separate, independent analyses were then performed for each variable (i.e., each frequency band from a particular area of the cortex) in each behavioral stage and at each exposure level In each analysis, one-way ANOVA followed by Dunnett's post hoc test was used to compare the control value to the data for the first exposure, second exposure, and postexposure hours. These comparisons were considered significant at $p<0.05$.

RESULTS

Effects of 2-h Exposure to Ethanol Inhalation on Sleep-Wake Cycle

Measurement of hourly durations of W, NREM and REM sleep during control days showed that NREM sleep dominated during 4-h recording period (Fig. 1). This baseline pattern was changed during exposure to ethanol at the minimal level of 101 ppm. During h-1 exposure a significant increase in W with a decrease in REM sleep was noted. Though the duration of different stages was not altered during h-2 exposure, the REM was increased during the postexposure period. Exposure to 397 ppm caused an increase in W during h-2 exposure and postexposure periods and a decrease of REM sleep during both h-1 and h-2 exposures and postexposure periods, NREM sleep decreased only in the postexposure period. At 804 ppm and 1610 ppm the stages were not significantly changed at any time during exposure and postexposure periods.

EEG Power Spectrum

Power spectra of EEG samples recorded from the somatosensory and visual cortices during sleep-wake stages indicate some qualitative and quantitative differences during W, NREM and REM sleep (Fig 2) The spectral power derived from the visual cortex was higher compared to that of the somatosensory cortex during all the stages. The W was associated with less power compared to that of NREM sleep. In NREM sleep predominant power was noted from both the cortices at δ and θ frequency bands and then gradually diminished in 13–20 (beta) Hz range. REM sleep was associated with predominant θ frequency band power at the visual cortex. The powers of the other frequency bands in REM sleep were lower compared to that of NREM sleep as in the W stage. In the somatosensory cortex during REM sleep all the frequency bands showed less power compared to those in NREM sleep, but the θ activity was not prominent like that of the visual cortex. Hence W and REM sleep cannot be distinguished from power spectral analysis of the somatosensory cortex (Fig. 2).

Power spectra in EEG recorded from the visual cortex or somatosensory cortex during W, REM and NREM sleep were not changed significantly due to exposure to ethanol inhalation at any concentrations used. Figure 3 shows the power spectra in the somatosensory cortex in different stages of sleep-wake cycle during exposure to ethanol inhalation at 4 different concentrations.

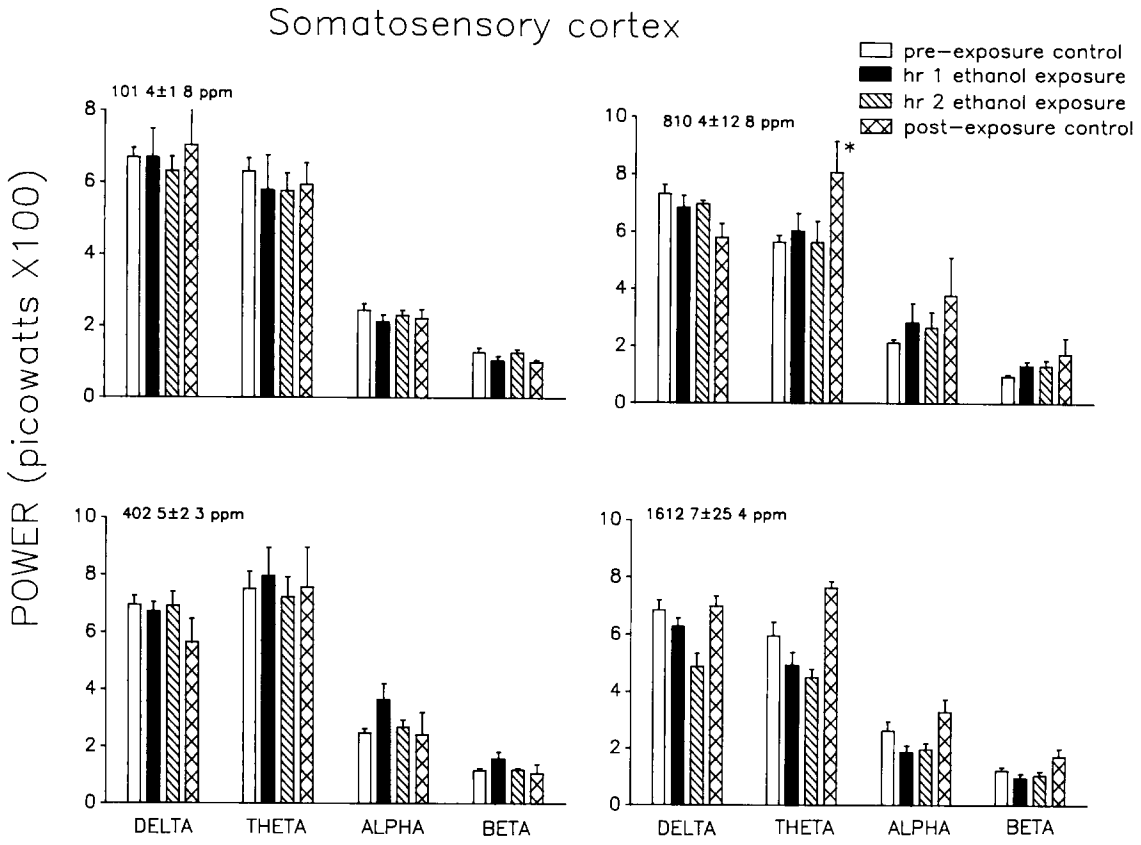

FIG 3 Lack of effects of ethanol inhalation at 4 different concentrations used on the power spectra in EEG recorded from the somatosensory cortex at different stages of the sleep-wake cycle

DISCUSSION

Exposure to ethanol inhalation at low concentrations (101 and 397 ppm) caused a dose-dependent increase in duration of a wake stage and a decrease in REM sleep; higher concentrations used did not show any effect. This would indicate an arousal reaction during exposure to ethanol at low concentrations. The observation on effects of ethanol on sleep-wake cycle can be supported by reports of generalized low-voltage, high-frequency activity particularly in the frontal cortices and limbic structures in animals at low doses of ethanol (9, 10, 14) However, as mentioned earlier (2), there is a great deal of variation of ethanol effects at low doses However, the power spectra of EEG as recorded from the somatosensory or visual cortex have shown a lack of sensitivity to the concentrations of ethanol used in this study. In our previous study (4), EEG power spectra, compared to stages of sleep-wake cycle, have also been shown to be less sensitive to toluene.

On the other hand, operant behavioral schedules have been more sensitive to ethanol inhalation It caused a decrease in the reinforcement rate at 102 ppm and higher concentrations in a fixed-ratio liquid-reinforced behavior, but at much higher concentrations (e.g , 603 ppm and above) in self-stimulation behavior (5). Thus comparison of data for ethanol effects on behavior (5) and EEG (current experiment) shows graded decrease of sensitivity to ethanol of the following parameters fixed-ratio liquid-reinforcement, duration of stages of sleep-wake cycle, self-stimulation behavior, EEG power spectrum, as was also observed in our xylene and toluene experiments (3,4).

In ethanol inhalation studies, blood concentrations of ethanol have been monitored and correlated with their pharmacological effects Thus mice showed ataxia, tremor and sleep with blood levels above 1 5 mg/ml, and coma above 3 5 mg/ml level (6) Physical dependence could be produced by maintaining the blood ethanol level at 2 or 1 mg/ml with the peak of withdrawal reaction progressively increasing between 6 to 9 days (7). In the behavioral study from our laboratory in rats (5), blood ethanol concentrations were found to be as low as 120 μg/ml to produce behavioral decrement in a fixed-ratio liquid-reinforced schedule Projecting the ethanol concentrations from that study to our present experiment it appears that a blood ethanol concentration as low as 110 μg/ml would produce the arousal effect on the sleep-wake cycle.

Our threshold for behavioral stimulation by ethanol exposure in respect to sleep-wake cycle (i.e., 100 and 397 ppm) appears to be lower than that set by OSHA (Occupational Safety and Health Administration). Permissible Exposure Limit (PEL) as well as Time Weighed Average (i e , the employee's average airborne exposure in any 8-h work shift of 40-h work week) for ethanol as set by OSHA is 1000 ppm or 1900 mg/m^3 (13).

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