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Hippocampal Rhythmic Slow Activity in Rat Lines Selected for Differences in Ethanol-Induced Motor Impairment

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KAHEINEN, P., E. R. KORPI, I. PYYKKÖ, S. MÄNTYSALO AND J. IGNATIUS. *Hippocampal rhythmic slow activity in rat lines selected for differences in ethanol-induced motor impairment.* PHARMACOL BIOCHEM BEHAV **30**(1) 177–181, 1988.—Hippocampal rhythmic slow activity (RSA) was recorded during rotation and vibration stimulation after saline and ethanol (2 g/kg) administration in restrained alcohol-sensitive (ANT) and alcohol-insensitive (AT) rats implanted with chronic bipolar electrodes in the dorsal hippocampus. The saline-treated ANT rats had more high-frequency RSA than the AT rats, especially during the rotational stimulation of the optovestibular mechanisms. The difference was not found during ethanol sessions. Plasma corticosterone levels were significantly higher in the AT than the ANT rats after the recording sessions. This first electrophysiological demonstration of an alcohol-sensitivity difference in the brain between these rat lines is discussed in relation to behavioral tilting plane test used in the development of the lines, to the different innate responses of the lines to acute stress, and to the plausible line differences in brain GABAergic and serotonergic mechanisms that are known to modulate hippocampal EEG in rodents.

Hippocampal EEG Alcohol sensitivity Selected rat lines Corticosterone

THE hippocampus participates in several important functions of the brain, including those in memory processes, alertness reactions and the handling of sensory, emotional and motor information [2, 7, 16]. Its functions *in vivo* can be monitored via implanted electrodes to record hippocampal electroencephalographic activity (EEG). A specific feature of the hippocampal EEG is a rhythmic slow activity (RSA), which is often called the hippocampal theta rhythm. Its frequency ranges from 4 to 12 Hz. The RSA can be behaviorally produced by arousal from various environmental stimuli [7]. It is also associated with voluntary movements [23] and rapid eye movement sleep [13,14]. Both behavioral and pharmacological manipulation can distinguish two types of RSA. Type 1 activity has a higher frequency (6–12 Hz) and occurs especially during voluntary movements; it is resistant to anticholinergic atropine, but it is sensitive to anesthetics, like ethyl ether and ethanol [11,25]. Type 2 activity has a lower frequency (4–8 Hz), occurs during immobility or reflex movements, is sensitive to atropine, but not to most anesthetics.

In the present study, we compared the hippocampal EEG of two rat lines selectively out-bred for high (ANT, Alcohol Non-Tolerant) or low (AT, Alcohol Tolerant) sensitivity to acute ethanol administration [6]. Although these rat lines differ significantly in behavioral tests for motor impairment after ethanol or other sedative drugs [9,21], no studies had previously been conducted in living animals to establish a neurophysiological basis for the line difference. We report here the effects of ethanol and various sensory stimulations on the EEG from the dorsal hippocampus of AT and ANT rats.

METHOD

Animals

Altogether 16 adult male rats (380–520 g) were used in the study: 8 ANTs and 8 ATs of the F_{26} generation. They were maintained in stainless steel wire cages in groups of 4 to 6 animals with artificial lighting from 6 a.m. to 6 p.m. The animals had free access to R3 rodent pellet food (Ewos AB, Södertälje, Sweden) and tap water. At least one month prior to the surgery and electrophysiological experiments, the motor-impairing effect of ethanol was tested in these rats with the tilting plane test 30 min after an intraperitoneal injection of ethanol (2 g/kg, 12% w/v solution in saline) [9].

Implantation of Hippocampal Electrodes

The rats were pretreated subcutaneously with atropine

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(0.05 mg/kg, Atropine^R, Orion Corporation Ltd., Finland), and then anesthetized with halothane (Halothan, Hoechst, W. Germany) for the duration of the operation. About one ml of lidocaine-adrenaline (both at 10 mg/ml) solution was infiltrated into the skin covering the skull, and the animal was placed into a Kopf stereotaxic frame, and implanted with two stainless steel wires (diameter 0.19 mm) insulated with metal varnish and trimmed to form a bipolar electrode with a tip separation of 1 mm. Implantation coordinates to the dorsal hippocampus bilaterally were 5 mm caudally from bregma, 3 mm laterally from midline, and 3 mm down from the surface of the dura according to the atlas of Paxinos and Watson [17]. The electrodes were secured to the skull with dental acrylic and four stainless steel screws, two of them extending down onto the dura through T-shaped burr holes.

After the operations the animals were immediately placed in individual plastic cages with floors covered with aspen chips, and given at least 3 days to recover from surgery before any experiments. On the first 2 days they were given injections of penicillin G procaine subcutaneously (0.1 ml/kg; 300,000 I.U./ml, Orion Corporation Ltd.).

EEG Measurements

Two or three days before the experimental sessions the animals were habituated with the test equipment and sensory stimulations without alcohol administration. All the measurements were carried out in a sound-attenuated, electrically-sealed room, where the devices could be remotely operated from an adjacent room.

The EEG recording and stimulation equipment and data collection and analyses methods are described in detail by Pyykkö *et al.* [18]. Briefly, the animals were held restrained in a Plexiglas cylinder with their heads secured outside the tube for EEG cables. The head of the animal was placed in the center of a turntable (diameter 50 cm), and the EEG signals from one hippocampus were taken through mercury slip rings to an amplifier (Siemens-Elema Mingograf EEG 10). The turntable was encircled by a black-and-white striped plastic cloth (optokinetic drum). A vibrating motor was fastened under the stomach of the animals with a Velcro harness inside the Plexiglas cylinder.

Each animal was tested after both an intraperitoneal injection of ethanol (2 g/kg as a 12% w/v saline solution) or an equivalent volume of saline, the order being randomized, with at least a three-day separation. The same AT and ANT animals were always paired for saline and ethanol sessions. Five min after the injection, the rats were restrained and allowed to calm down for 10 min. The baseline EEG was first recorded for five min, after which the turntable was rotated by a DC motor twice to the right and to the left (acceleration 12°/s^2 until maximum speed of 180°/sec was achieved at about 15 sec, deceleration took 25 sec) with 45 sec intervals. At 35 min, the vibration stimuli were delivered at frequencies of 50, 100 and 150 Hz (5, 10, and 15 V) for 10 sec with 30 sec intervals. The highest frequency that gave the most consistent responses was repeated four times. At the end of the experiments the baseline EEG was again recorded for 5 min. Tail tip blood samples (100 μl) were drawn for ethanol determinations [5] just before the first and just after the last baseline recording. At the end of the experiments, 200 μl of blood was collected into heparinized microtubes, plasma separated and corticosterone levels measured with a radioimmunoassay using a corticosterone antiserum B3-163 according to the supplier's manual (Endocrine Sciences, Tarzana, CA).

The EEG records obtained from the amplifier were stored on magnetic tape (Racal Thermionic) and later analyzed with a computer (Digital PDP 11/23). The time constant of the EEG amplifier was set at 0.3 sec and the cut-off frequency at 15 Hz. After analog-digital conversion the digitalized signal was displayed on a screen for editing purposes and samples of baseline recordings and responses to sensory stimuli stored on a hard disc. An EEG analysis program made Fourier transformations and calculated the spectral densities of four frequency bands (1–4, 4–8, 8–13, and >13 Hz). The percentage distribution and energy in the frequency bands were the main parameters used in the analysis. Then the energies of the two RSA-frequency bands (4–8 and 8–13 Hz) were summed to show the strength of the response to sensory stimuli, and the ratios of the energies of these two channels were calculated to show the relative frequency distribution of the RSA response in low and high-frequency bands. The low and high-frequency bands were assumed to represent the Type 1 and Type 2 RSA activities, although no behavioral and pharmacological characterizations could be done in the present study.

Histology

After the experiments the animals were decapitated and the brains quickly frozen inside a Tissue Tek^R block in isopentane/solid CO_2 bath. The blocks were then sectioned with a cryostat at 30-μm intervals, stained with thionine blue, and finally the serial sections examined with a light microscope to locate the lower tips of the implanted electrodes.

RESULTS

Visual Analyses

Microscopic examination of the electrode tips revealed them to be in the dentate gyrus of the hippocampus or slightly above it in all rats of both lines. No systematic differences were observed in the electrode locations between the lines.

Visual examination of the EEG's did not show any clearly visible difference between the AT's and ANT's. The baseline EEG in both lines displayed mixed-frequency activity in both saline and ethanol experiments; during presentation of rotation and vibration stimuli the EEG was synchronized (Fig. 1) in all cases except one (ANT). This one ANT rat displayed a reverse phenomenon, where relatively high-amplitude baseline activity was greatly attenuated both during rotation and vibration. It was also obvious that during several ethanol experiments with both rat lines, the rotation and vibration stimuli had an alerting effect, abolishing slow high-amplitude delta waves (1–4 Hz) for a period longer than the stimulation was given to produce RSA. There was no clear sign of habituation occurring in the course of the five 10-sec vibration stimuli at the highest vibration frequency (150 Hz).

Quantitative Analysis

There were only minor differences between the lines in the frequency profiles of hippocampal EEG analyzed by a computer. The ANT animals had more frequencies over 13 Hz (as % of the total EEG power) during the baseline recordings before and after the stimulations and during the vibration stimuli. This difference was significant ($p<0.05$) only after saline injections (data not shown).

The main attention of the analyses was focussed on the hippocampal RSA, analyzed here in frequency bands of 4–8

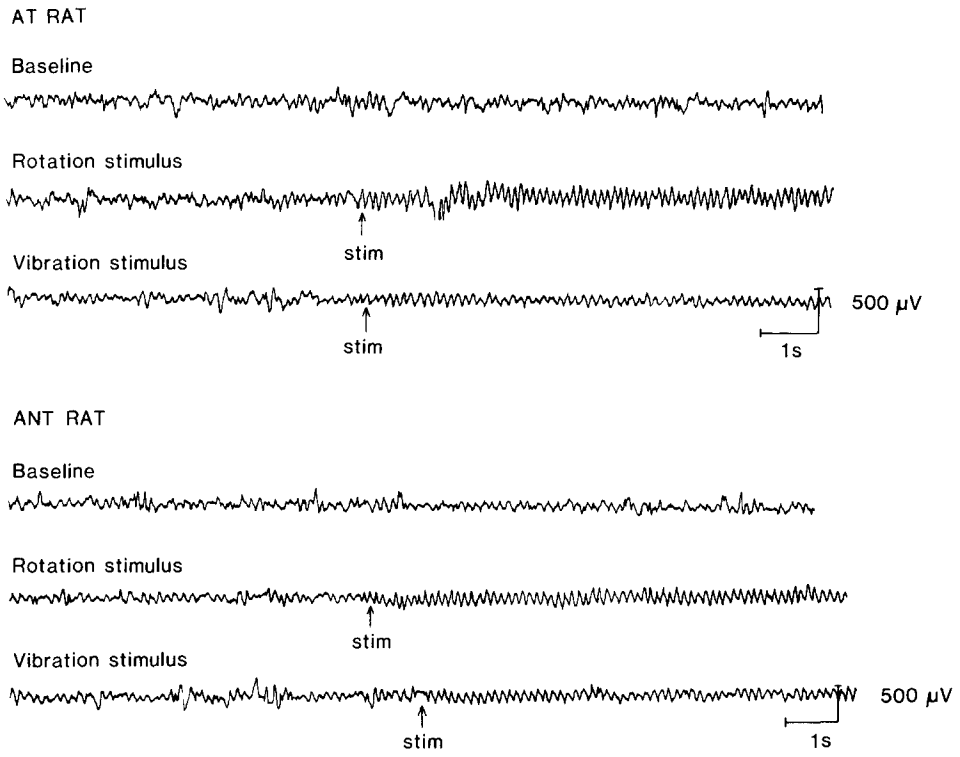

FIG. 1. Representative hippocampal EEG recordings from an AT and an ANT rat after saline pretreatment. The vibration stimulus was at 150 Hz. EEG was recorded using a high-frequency filter at 15 Hz, a time constant of 0.3 sec, and a sensitivity of 500 μV/10 mm.

and 8–13 Hz. The total power of the EEG estimated by a computer in these bands was similar in AT and ANT rats. However, when the ratios of the powers in the two RSA bands were compared (to get rid of the interanimal variation in the baseline power of the EEG), it emerged that ethanol highly significantly ($p<0.001$) decreased the proportion of the high-frequency band in the ANT animals (Fig. 2). Moreover, the AT's had lower ratios during both rotational (paired Student's t-test: $p<0.002$) and vibration ($p<0.02$) stimulations after saline treatment than the ANT's, i.e., the AT's had more RSA of the lower frequency. The main action of a moderate dose of ethanol was noted to be a relative reduction of higher frequency RSA rhythm in the alcohol-sensitive ANT line.

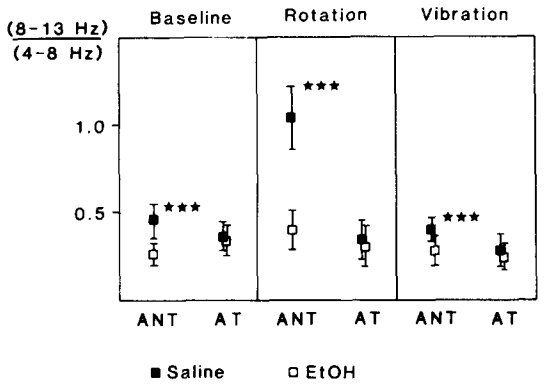

FIG. 2. Ratio of the powers of high-frequency RSA to that of the low-frequency RSA in AT and ANT rats. Statistical significance of the differences between the saline- and ethanol-treated animals within the lines were assessed by paired Student's t-test (n=8): ***$p<0.001$ (mean±SD).

Blood Ethanol Levels

No significant differences between the AT and ANT lines were found in blood ethanol concentrations in the tilting plane test or in the sessions of hippocampal EEG recordings (Table 1). Ethanol concentrations were slightly more variable during the recording sessions while the animals were restrained than during the tilting plane test when they could move freely.

Plasma Corticosterone Levels

Plasma corticosterone concentrations were fairly high in both rat lines during experimental sessions (Table 2). They were significantly ($p<0.01$) higher than the concentrations for another group of naive rats taken directly from their home group cages for tail blood sampling. In the experimental groups, the AT's had significantly higher corticosterone concentrations than the ANT's. Ethanol administration decreased the corticosterone levels by the same amount in both lines but the decrease was statistically significant ($p<0.02$) only in the ANT's.

DISCUSSION

The present results provide the first evidence for a neurophysiological difference in ethanol sensitivity between the

TABLE 1

BLOOD ETHANOL CONCENTRATIONS IN AT AND ANT RATS DURING THE TILTING PLANE TEST AND DURING REGISTRATIONS OF HIPPOCAMPAL EEG

	Ethanol (mmol/l)	
	AT	ANT
Tilting plane test, 30 min	47.2 ± 5.3	46.2 ± 2.6
EEG recording, 15 min	28.0 ± 9.5	26.6 ± 12.7
EEG recording, 45 min	34.8 ± 7.6	34.2 ± 7.5

Values are means ± S.D.s, 8 rats in each group.

TABLE 2

PLASMA CORTICOSTERONE CONCENTRATIONS IN AT AND ANT RATS AFTER REGISTRATION OF HIPPOCAMPAL EEG UNDER RESTRAINT CONDITIONS AND IN NAIVE NON-RESTRAINED CONTROL RATS

	Corticosterone (μmol/l)	
	AT	ANT
Naive rats (5)	0.88 ± 0.21	0.79 ± 0.20
EEG recording sessions		
Saline (8)	1.60 ± 0.22	1.30 ± 0.21*
Ethanol (8)	1.46 ± 0.20	1.16 ± 0.14†‡

Values are means ± S.D.s (number of animals). Statistical comparisons were carried out using paired Student's *t*-test: *$p<0.03$ between the lines; †$p<0.005$ between the lines; ‡$p<0.02$ between saline and ethanol experiments.

rat lines selectively developed by out-breeding for high and low ethanol sensitivity. We detected an ethanol-sensitive component of the hippocampal EEG in the ANT rats. Although several neurochemical differences exist between these lines, they have not provided any clear clues to the inherited mechanisms of ethanol sensitivity [3, 8, 10]. The importance and meaning of the present finding thus can be discussed in relation to known facts about the regulation and pharmacology of hippocampal EEG.

The main difference between the AT and ANT lines was observed during the rotation stimulation (Fig. 2). The hippocampus has been proposed to be important in the orientation of the animal in space [15]. The observed EEG line difference might be associated with the behavioral line difference in ethanol-induced motor impairment measured in the tilting plane test, since the test requires rapid adjustment of posture to the changing position of the floor. Rotation stimulation in our experiments activated both vestibular and optokinetic responses, which are important also in the tilting plane test performance, among other mechanisms needed for motor coordination. Thus the present finding could be functionally related to the difference the genetic selection has created between the lines. It has been shown that the frequency of the hippocampal RSA is highest at the initiation of movements [23, 24, 27]. If the ANT rats require higher-frequency hippocampal functioning for orientating themselves during rotation (and during the tilting plane test) than the AT rats, they might become more sensitive to the ethanol-induced motor impairment, when ethanol depresses this component of the RSA response in them.

Ethanol produces hypothermia which also is associated with decreased RSA frequency [26,27]. In the present study, ethanol slightly decreased the ratio of the high and low frequencies of the baseline RSA in the ANT rats. The ANT rats, however, little differ from the AT rats in their hypothermic response to a large dose of ethanol (4 g/kg, IP) [4]. It is thus unlikely that the ethanol-induced hypothermia would explain the line differences found, especially not that found during the rotation stimulation.

It is, however, possible that our findings are related to the specific experimental design we used or to accidental genetic drifts produced in the course of the selection. We carried out the recordings in restrained animals. The stress resulting from this procedure could be demonstrated by increased plasma corticosterone concentrations. Corticosterone has been shown to decrease the frequency of the minimal current of septal stimulation to drive hippocampal RSA in rats [22]; therefore, the observed difference in corticosterone levels may have contributed to the greater proportion of high-frequency RSA in ANT rats than in AT rats (Table 2). The effect of ethanol on RSA of the ANT rats cannot be explained by differential corticosterone concentrations.

The greatest difference in RSA frequency distribution was found in the saline experiments and ethanol administration actually abolished the difference. During the selection, it has been a goal to avoid any baseline motor coordination difference by making sure that the performance of the both lines is similar in the tilting plane test without alcohol. Recently, however, Sinclair *et al.* [20] found that the naive AT rats are more active in a short, low-stress swimming test than the naive ANT rats. If the AT rats attempted to struggle within the restraining cylinder more than the ANT rats, the share of the high-frequency RSA should have been greater in them than in the ANT rats [23]. But it was the ANT line, however, that showed the greater proportion of high-frequency RSA without alcohol. Thus it is difficult to make any conclusion about the possible confounding effects of struggling during the recordings, since we could not monitor the muscular activity while recording the EEG. After administration of ethanol the proportion of the high-frequency RSA decreased in the ANT rats to the same level found in the AT rats, possibly due to the sensitivity of the high-frequency RSA to ethanol [25] and/or to muscle relaxation.

The neurochemistry of hippocampal RSA is poorly understood, especially that of the high-frequency, ethanol-sensitive activity. The decrease by excess corticosterone of the septal stimulation frequency giving the RSA response at the lowest (threshold) current [22] can be altered by modifications of the serotonin system in a way that suggests either that corticosterone may act by inhibition of the serotonergic system [22] or that serotonin and corticosterone act on a common or related neuronal mechanism [1]. On the other hand, our rat lines can be distinguished behaviorally by GABAergic drugs in the same way as by ethanol [9,21]. The GABAergic drugs and ethanol characteristically flatten the septal driving curve [12,19]. Without further pharmacological experiments it is still premature to postulate any role for either the inhibition of the serotonergic mechanisms or the enhancement of the GABAergic mechanisms to explain the line difference in the hippocampal EEG observed in the present study.

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