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Interaction of Self-Stimulation and Ethanol-Intake Behaviors in Rats

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GALLARDO-CARPENTIER, A. AND S. N. PRADHAN. *Interaction of self-stimulation and ethanol-intake behaviors in rats.* PHARMAC. BIOCHEM. BEHAV. 11(4) 413–417, 1979.—The effect of ethanol (10% v/v) intake was studied on the rate of self-stimulation (SS) in rats implanted with bipolar electrodes in the posterior hypothalamus. The rats were divided into 3 groups: in Group A, 6 rats were trained to press a bar for SS, and allowed to drink water only; in Group B, 6 rats were trained also for SS, and in Group C, 5 rats were put on to rotarod performance and served as controls for the physical exercise incurred in SS schedule; rats of both Groups B and C were offered ethanol and water in free choice. At the termination of the experiment, the contents of norepinephrine (NE) and serotonin (5-HT) in diencephalon-midbrain (DM) and pons-medulla (PM), and dopamine (DA) contents in DM and caudate nucleus (CN) of these rats were estimated. While in Group A rats the SS rate decreased gradually, in Group B rats the SS rate increased significantly and their ethanol intake increased to approximately 60% of their total fluid intake (TFI). In Group C rats ethanol intake was low (about 30% of TFI), compared to Group B. Neurochemical studies in Group B and C showed increased NE and 5-HT contents in DM and PM and decreased DA contents in CN and DM, compared to controls. The interaction between the two reinforcing behaviors seems to be related to induced changes in the brain amines.

Rats Ethanol Self-stimulation Reinforcing behavior Drinking behavior Neurotransmitters Norepinephrine Dopamine Serotonin

INTRACRANIAL self-stimulation (SS), as demonstrated first by Olds and Milner [16] in rats, appears to provide a suitable animal model for pleasure-seeking behavior associated with motivation and drive. This behavior has been shown to be modulated by a number of neurotransmitters including norepinephrine (NE), dopamine (DA), serotonin (5-HT) and acetylcholine (ACh) [21].

On the other hand, certain chemicals because of their apparent stimulating or euphoric effects, induce drug-seeking or drug-taking behavior in man and animals. Ethanol, a good example of this group, has been shown to induce self-administration behavior in animals and drug-taking behavior in man leading to psychological and physical dependence. Ethanol also produces some dose- and time-dependent effects on several behaviors [19]. At low doses, it stimulates motor activity [7], food-reinforced operant behavior [27] and SS [8,25]; at high doses some of these behaviors are depressed.

The metabolism of biogenic amines is also affected by ethanol, although the findings have been variable [15]. Whereas para-chlorophenylalanine (PCPA, a 5-HT synthesis inhibitor), would produce aversion to ethanol [15], higher content of 5-HT has been found in the brain of ethanol-preferring rats than in that of nonpreferring animals [7,18]. The greatest elevation of 5-HT activity occurs in the hypothalamus, thalamus and midbrain of ethanol-drinking rats [2]. Ethanol also causes increase [18], decrease [3, 13, 24] or no change in NE levels [20] following its chronic administration. The plasma dopamine-β-hydroxylase (DBH) activity has been shown to correlate with mood state in volunteers drinking ethanol [11], and DBH inhbitors suppresses ethanol ingestion in Wistar rats [4], indicating that the intrinsic ratio of DA and NE in the brain may be an important modulating factor in ethanol intake [15].

The purpose of the present experiment was therefore to study the interaction between the two reinforcing behaviors, e.g., SS and ethanol intake, in rats by measuring the rate of responding in self-stimulating rats with hypothalamically implanted electrodes and allowed to drink ethanol ad lib. The levels of biogenic amines in discrete brain areas were also assayed at the completion of the experiment for correlation with behavioral changes.

METHOD

Male Wistar rats with initial weights of 300–390 g were maintained on food (Purina Lab chow pellets) and fluid ad lib. Each animal was housed separately from the beginning of the experiment.

Behavioral Procedures

The rats were stereotaxically implanted, under pentobarbital (50 mg/kg, IP) anesthesia, in their posterior hypothalamus, with a set of bipolar stainless steel electrodes (0.01 in. in diameter insulated except at the tip, from Plastic Products, Inc., Roanoke, VA). The stereotaxic coordinates were 3.5 mm posterior to bregma, 0.8 mm lateral to the midline and 9 mm in depth from the top of the skull. The electrode placement was confirmed histologically in some repre-

sentative rats at the completion of the experiment, according to the atlas of König and Klippel [12].

On the bases of the type of fluid intake and behavioral scheduled involved, the rats were divided into three groups: in Group A, 6 rats were trained for SS schedule and provided with water; in Group B six other rats under SS schedule were provided with ethanol solution (10% v/v in water) in addition to water; in Group C five rats were given both water and ethanol solution (as in Group B), but were subjected to forced exercise while climbing a rotating rod and served as controls for physical exercise incurred in the SS schedule. For such rotarod performance, a wooden rod of 1 in. diameter was rotated at a speed of 8 rpm. If a rat would fall down from the rod, it was again put on the rod during the session lasting for 30 min a day.

For SS schedule, details of which have been described earlier [22], rats after recovery from surgery, were trained to press a lever in a Skinner box for reinforcement with intracranial electric stimulation. Each lever press provided a stimulation train of 0.4 sec duration, the stimulus being a sine wave of 60 Hz. The current intensity (as rms) remained constant at slightly above the threshold level throughout the sessions, and varied from rat to rat from 25 to 90 μA. The threshold for each rat was considered to be a minimum current able to generate 1 to 10 responses per minute in excess to that observed in the absence of current.

When the performance of the rats under SS schedule became stable, their control level of daily water intake was measured for a week. During the following experimental period (6 weeks), the rats of respective groups were subjected to a 30-min behavioral session daily for 5 days a week. SS responses were recorded every 10 min during the session.

Water and ethanol solution were provided in 100 ml Kimax lab test tubes and positions of the tubes in the cage were rotated at random. Water intake in Group A rats and water and ethanol intakes in Group B and C rats were measured daily. Their body weights were recorded once weekly to monitor their general conditions.

Biochemical Procedures

For estimation of ethanol concentration, blood samples were drawn from the tail vein of rats at 9:00 a.m. and chemical analysis was done by the micro-method of Williams *et al.* [28].

Estimation of brain neurotransmitters was done at the end of 6 weeks of the experimental period. Rats were sacrificed by exposure to microwave radiation and different brain areas such as the caudate nucleus (CN), the diencephalon-midbrain (DM) and the pons-medulla (PM) were dissected out at 4°. DM and PM were assayed for NE and 5-HT, and DM and CN were assayed for DA. The tissues were homogenized in ice cold 0.4 N perchloric acid (100 mg/ml), centrifuged in a Sorvall (RC 2-B model) at 4° for 20 min at 10,000 rpm. NE, DA and 5-HT were extracted simultaneously from the supernatant of the tissue homogenate according to the method of Cox and Perhach [10]. NE and DA were assayed by the method of Chang [9] and Spano and Neff [23]. 5-HT was extracted and determined according to the procedure of Ansell and Beeson [6] and Maickel *et al.* [14].

Data Analysis

In both behavioral and neurochemical experiments, the data of each parameter from several rats at a particular experimental condition were used to calculate the mean (± SE). Student's *t*-test was performed to evaluate the statistical significance of the change from the control. The data were further analyzed by using appropriate ANOVA tests [29]. Drug effects were calculated in terms of percent change from the controls in neurochemical and some behavioral experiments.

RESULTS

Self-Stimulation

SS respondings of the rats of the water-intake as well as the ethanol-intake groups varied from 3500 to 4000 per 30 min during the initial period. During the next 6 weeks, the respondings of the rats in the water-intake group decreased, while those in the ethanol-intake group increased. The time-courses of the changes in SS responding of representative rats, one from each group, during the 6-week period are shown in Fig. 1. During ethanol ingestion, SS of Rat 3 increased significantly, $F(4,20)=9.01$, $p<0.005$. Self-stimulation on Weeks 1 and 2 was not different from control (Week 0 data), $F(2,8)=2.51$ and $F(2,8)=2.74$, $p>0.10$, but on Weeks 4 and 6, SS was significantly higher: $F(3,8)=4.58$ and $F(4,8)=15.86$, $p<0.05$ and $p<0.005$, respectively. On the other hand, SS of Rat 26 did not change, $F(4,20)=1.16$, $p>0.10$. The mean percent changes of SS respondings in rats of both groups from their respective initial controls are presented in Fig. 2. Analysis of the SS data over weeks with a two-factor ANOVA with repeated measures over weeks revealed a significant group effect, $F(1,10)=8.12$, $p<0.025$. Multiple comparison tests using the appropriate MS error term from the overall ANOVA indicated no group difference on Week 1 ($p>0.10$) but significant group differences ($p<0.005$) on Weeks 2, 4 and 6.

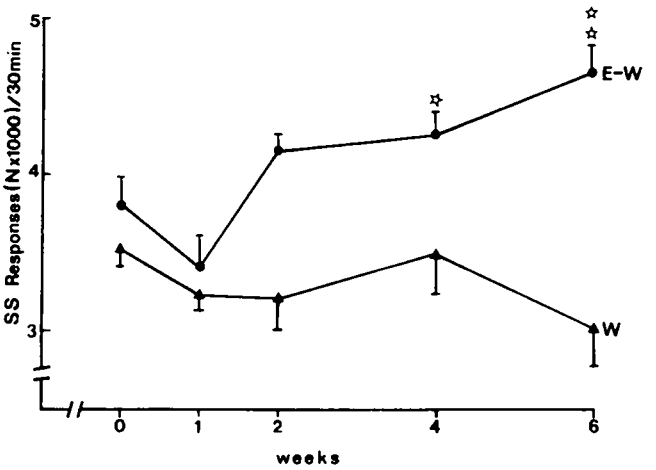

FIG. 1. Self-stimulation (SS) responses of representative rats, one from each of the two groups: A—rat drinking water ad lib (▲, Rat 26); B—drinking ethanol and water in free choice (●, Rat 3). Each value represents the daily mean ± SE for a given week. *Significantly ($p<0.05$) different from the control. **Significantly ($p<0.005$) different from the control.

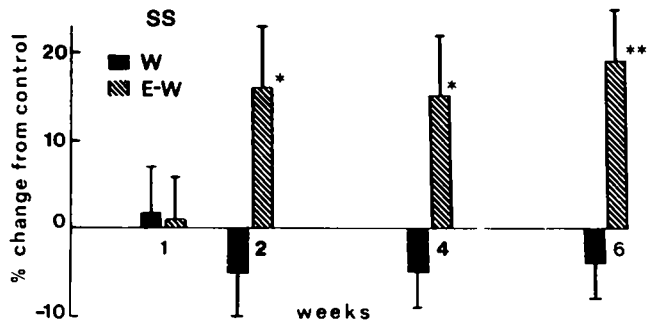

FIG. 2. Time-course of changes (percent of controls) in SS responding in two groups of rats: Group A—rats (N=6) drinking water ad lib; Group B—rats (N=6) drinking water ad lib during the initial control period followed by water and ethanol (10%) in free choice during 6 weeks of experimental period. Significance of difference from control: $*p<0.05$, $**p<0.005$.

TABLE 1

ETHANOL, WATER AND TOTAL FLUID INTAKE IN SELF-STIMULATING AND CONTROL RATS

Rat Group	Experimental Condition	No. of Rats	Fluid Intake* (ml/100 g B.W./day) Total	Water	Ethanol
A	SS	6	6.1 ± 0.3	6.1 ± 0.3	—
B	During SS	6	7.0 ± 0.2	2.9 ± 0.4	4.1 ± 0.2
	Post SS	3	6.9 ± 0.1	2.3 ± 0.2	4.6 ± 0.2
C	Rotarod Performance	5	6.3 ± 0.2	4.6 ± 0.3	1.8 ± 0.3

*Average of the daily fluid intake during the experimental period.

Water and Ethanol Intake

During the control period intake of water offered ad lib in Group B rats was 7.8 ± 0.6 ml/100 g body weight/day. During the following 6 weeks when the rats were subjected to the daily SS schedule and given the opportunity to drink ethanol solution in addition to water, ethanol solution comprised approximately 60% of their average daily total fluid intake (TFI) over the period (Table 1). Throughout these 6 weeks their water intake decreased progressively, causing a significant difference between water intake during the first and last weeks. During these 6 weeks the ethanol intake increased, but not significantly. However, the decrease of water intake was comparatively more than the increase of ethanol intake, so that the TFI decreased progressively with a significant ($p<0.05$) difference between the intakes during the 1st and 6th weeks (Fig. 3). When 3 rats from Group B were maintained on both water and ethanol for 5 more weeks without being subjected to SS schedule, the same pattern of fluid consumption with high ethanol intake persisted, as shown in Table 1. On the other hand, the ethanol intake in the rats performing the rotarod schedule, remained low (about 30% of TFI) and water intake was high compared to those in the self-stimulating group (Table 1).

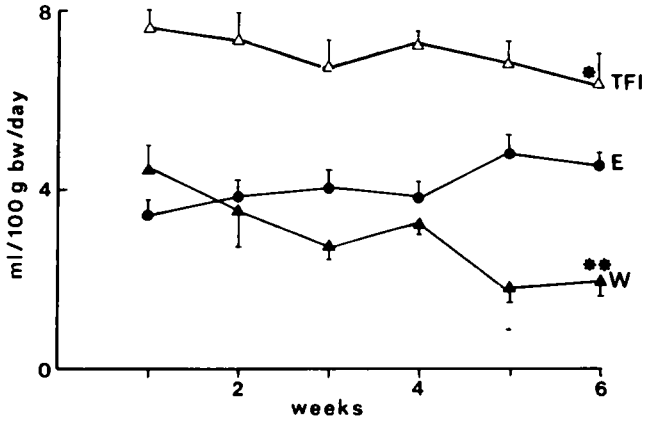

FIG. 3. Time-courses for intake of ethanol (●), water (▲) and total fluid (△) in 6 rats of Group B, under a schedule of self-stimulation of the PH area for a 6-week period. Each point represents the mean ± SE of fluid intake of 6 rats per day in a given week. Significance of difference from the fluid intake of the first week: $*p<0.05$; $**p<0.01$.

Blood Ethanol Concentration

In the control blood samples of rats taken in the morning no ethanol was detected. Following 6 weeks of ethanol intake the average concentration of blood ethanol was found to be 0.16 g% (ranging from 0.10 to 0.25 g%) in 5 out of 6 rats. In the remaining rat, three blood samples collected on different days were negative.

Brain Neurotransmitters

Table 2 shows the concentrations of three neurotransmitters in the discrete brain areas in different groups of rats. NE concentrations in the DM, and 5-HT concentrations in the PM and the DM increased significantly, and DA concentrations in the CN and the DM decreased significantly in both the rotarod and the SS groups of rats. There was no change in the NE concentration in the PM in either group.

DISCUSSION

In the present experiment SS responding rate was increased in rats drinking a 10% solution of ethanol and water ad lib, while it decreased in rats drinking water only, showing that ethanol was involved in this rate-increasing response. Blood concentration of ethanol in five out of the six rats was low (0.1–0.25 g%). Other studies [8,25] also showed increased responding in rats treated with low IP doses of ethanol. In these reports the blood ethanol concentration, although not estimated, may be assumed to be low. On the other hand, in the present experiment the ethanol intake of the hypothalamic SS rats was increased, and this effect was persistent even when SS was stopped. Increased preference for alcohol to water has also been demonstrated following hypothalamic electric stimulation in rats and such change was also persistent [5,26].

As mentioned above, blood ethanol concentrations in rats of this experiment were low. Such low concentration in the morning (9:00 a.m.) blood samples of ethanol drinking rats may be explained by the fact that rats are nocturnal animals

TABLE 2

BRAIN NEUROTRANSMITTER LEVELS IN CONTROL, ROTAROD AND SS GROUPS OF RATS

Neurotransmitters§	Brain Areas*	Concentration (ng/g)† Controls	Rotarod Group	SS Group
NE	PM	674 ± 12	659 ± 27	697 ± 33
	DM	479 ± 05	576 ± 32‡	610 ± 19§
DA	CN	2,481 ± 98	1,859 ± 16§	2,009 ± 63‡
	DM	211 ± 02	135 ± 14§	94 ± 08§
5-HT	PM	600 ± 09	936 ± 62‡	935 ± 44§
	DM	681 ± 05	822 ± 34‡	739 ± 18‡

*NE, Norepinephrine; DA, Dopamine; 5-HT, Serotonin; PM, Pons-medulla; DM, Diencephalon-midbrain; CN, Caudate Nucleus.

†Mean ±SE for rats in each group. Rats of SS and rotarod groups were subjected to self-stimulation schedule and rotarod performance respectively for 30 min/day. Both groups were provided with free access to water and ethanol. Rats in the control group were given water only

‡$p<0.01$.

§$p<0.001$.

and as such would drink more during the night rather than the morning, and that they metabolize ethanol at a very high rate [17]. It is therefore not surprising that in one of our rats ethanol could not be detected in the morning blood samples on different occasions, although it was a very heavy drinker.

The enhancement of SS rate after ethanol intake was associated with changes in the level of some neurotransmitters in discrete brain areas. The noradrenergic mechanisms has been shown to play an important role in SS by enhancing it [3]. In the present experiment the intake of a 10% solution of ethanol increased the NE content in the DM by 27%. This observation is corroborated by a 20% increase of NE in the telencephalon of alcohol-preferring rats reported by Penn *et al.* [18]. In studies involving DBH activity in rats [3] as well as in man [11] the ratio DA:NE was increased with a decrease in ethanol intake and vice versa. In rats of the present experiment, DA in the DM decreased, while NE in the same area increased, so the ratio between these two neurotransmitters decreased threefold, thus lending support to the importance of this ratio in the ethanol drinking behavior. A higher 5-HT level was also found in the DM of our rats, as also reported by others [1,18] showing also the involvement of serotonergic mechanisms in the drinking behavior. It thus appears that increase in hypothalamic SS responding in rats along with increase in their ethanol intake can be correlated to the changes in the neurotransmitter levels in the discrete brain areas.

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