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Ethanol Preference Following Hypothalamic Stimulation: Relation to Stimulation Parameters and Energy Balance[1]

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ATRENS, D. M., P. MARFAING-JALLAT AND J. LE MAGNEN. *Ethanol preference following hypothalamic stimulation: Relation to stimulation parameters and energy balance.* PHARMACOL BIOCHEM BEHAV **19**(4) 571–575, 1983.—Rats were given 5, 10, 20 and 30 min daily sessions of lateral hypothalamic stimulation. Approximately half of the rats showed a large and highly significant increase in their total intake of and preference for 10% v/v ethanol which was continuously available in their home cages. In terms of latency, total consumption and preference for ethanol, 10 min of daily stimulation produced a much greater enhancement than did 30 min. The ethanol drinking rats used more energy per unit of body weight which suggests that the stimulation and/or the ethanol itself may have increased energy expenditure. Simply changing the diet from powdered chow to identical composition pellets produced a large reduction in both total ethanol intake and preference. Reinstating the powdered diet produced a rapid reinstatement of ethanol drinking. These data are discussed in terms of ethanol's role in modulating stimulation induced changes in energy balance.

Lateral hypothalamus Electrical stimulation Ethanol Drinking Diet Energy balance Metabolism

A NUMBER of experiments have shown that electrical stimulation of the lateral hypothalamus can produce a long lasting enhancement of ethanol intake in the rat [1–5, 11–13]. This enhancement is reflected in post-stimulation increases in both the quantity of ethanol intake and preference for ethanol over water. However, the magnitude of this effect and its relation to procedural variables remains a matter of dispute. It is clear that in the experiments to date a large proportion of the enhancement of ethanol intake is a result of making ethanol available only on alternate days. Those experiments in which ethanol has been continuously available have reported the enhancement produced by the stimulation to be non-existent [8,14], small [13] or very slow in appearing [1]. If, as these studies suggest, the stimulation by itself produces only a very weak enhancement of ethanol intake, this paradigm would be of rather limited use in studying hypothalamic modulation of ethanol intake. Whether the relatively weak enhancement of ethanol intake produced by lateral hypothalamic stimulation is an inherent limitation or simply reflects the use of less than optimal parameters remains to be determined.

The studies which have reported the weakest enhancement effects have all used extreme stimulation parameters. For example, Martin and Myers [8] used 0.3 sec trains of stimulation and Amir and Stern [1] used 5 sec trains of stimulation. These studies respectively found no enhancement [8] and a very slow appearing enhancement [1]. At the other extreme, Wayner *et al.* [14] found no enhancement with 60 sec stimulation trains. All of the studies reporting a substantial enhancement effect have used 20 sec trains of stimulation [2–5, 11, 13]. Although the above studies differ significantly in terms of the method of stimulation presentation (forced or self-stimulation) as well as in electrode location and other variables, they nonetheless suggest some boundary conditions for obtaining the enhancement of ethanol drinking effect. In order to allow adequate temporal summation of activity in the neuron system which subserves the enhancement effect, the length of the stimulation trains should be greater than 5 sec, but less than 60 sec.

The above studies, however, provide little indication as to an optimal daily stimulation period. The greatest enhancement has always been found at 30 min [2–5, 11, 12] whereas

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longer sessions have generally resulted in smaller effects [8,14]. Shorter sessions have not been investigated. The present experiment is an analysis of the effects of daily stimulation session length on the enhancement of ethanol drinking effect. By making ethanol continously available, it is possible to evaluate the magnitude of the enhancement without the confounding influence of the commonly used alternate day availability procedure. Further, by measuring food intake it may be possible to relate these data to energy balance.

METHOD

Animals

This experiment used 20 male Wistar rats weighing from 223–255 g at the beginning of the experiment. The rats had free access to powdered lab chow in their home cages except for one 6-day period when they were switched to the same food in cube form. The lights were on in the experimental room from 600 to 1800 hours.

Apparatus

The home cages were 43 cm high, 25 cm diameter clear acrylic cylinders with perforated stainless steel floors. The powdered food was available from a deep dish mounted in a recess in the side of each cage. Two graduated cylinders, one containing tap water and the other 10% v/v ethanol were attached to the side of each cage. The fluids were presented through stainless steel drinking tubes whose openings were approximately 4 cm above the level of the floor. Their position was varied randomly throughout the experiment.

The cages in which the brain stimulation was administered were identical to the home cages except that they did not contain either a food cup or drinking tubes. The brain stimulation consisted of symmetrical, biphasic, 200 μsec, 50 Hz pulse pairs which were presented to the rat through a mercury commutator located on a swing arm above each cage. In each pulse pair, the cathodal pulse was presented first and the peak amplitude was 100 μa with the exception of one rat where convulsive-like motor effects dictated a reduction to 50 μa.

Procedure

Body weight as well as the intake of food, water and ethanol were measured daily for seven days before surgery. On the day of surgery all rats were anesthetized with Nembutal (45 mg/kg) and implanted with 254 μm diameter, stainless steel, monopolar electrodes (Plastic Products 0.010 SW) aimed for the lateral hypothalamic area. The coordinates relative to bregma with the skull in a flat position were: anterior 2.5, lateral 1.6 and ventral 8.5. The indifferent electrode was made of uninsulated stainless steel wire wrapped around a stainless steel screw (Plastic Products 0-80×3/32) which was located in the left front quadrant of the skull. The entire assembly was fixed in place with dental acrylic cement and two additional screws.

After the operation the rats were returned to their home cages and the experimental variables were measured daily for another week after which the rats were randomly assigned to 4 groups and the daily stimulation sessions were begun. The four treatment groups differed only in the length of the daily stimulation sessions (5, 10, 20 or 30 min). The stimulation was administered at the same time each day (11:00–14:00) and consisted of 20 sec pulse trains alternating with 20 sec intervals of no stimulation.

After 23 consecutive days of stimulation, the stimulation was discontinued and the dependent variables except for food intake were measured for a further 7 days. Following this, the food was changed to pellets with exactly the same composition as the powder used throughout the rest of the experiment. The pellets were the only source of solid food for the next 6 days. Ethanol and water intake were measured for only the final 4 days of the pellet diet and body weight on days 3, 5 and 6. After this the pellets were removed and the powdered diet was reinstated for the final 5 days of experiment.

At the conclusion of the experiment, the rats were killed with Nembutal and their brains were rapidly dissected and placed in 10% formal-saline. After approximately one month of fixation the brains were frozen to −20 and serial 80 μm horizontal sections were taken on a Leitz microtome and mounted on glass slides. The mounted tissue was stained metachromatically with Toluidine Blue 0 and the electrode positions were microscopically determined with reference to the atlas of Paxinos and Watson [9].

RESULTS

The electrode locations which are presented in Fig. 1 indicate that of the 20 electrodes, 17 were located in the lateral hypothalamic area at the approximate anterior-posterior level of the ventromedial hypothalamic nucleus. One electrode penetrated the base of the brain. Another was located in the amygdala and a third was located in the thalamus.

Of the 20 rats, 9 were classified as drinkers on the basis of their mean ethanol intake during the last half of the stimulation period exceeding 3 g/kg/day and being at least twice their mean pre-stimulation intake. Apart from the fact that all of the drinkers had their electrodes located in the lateral hypothalamus, there was little apparent anatomical differentiation of effects.

The data indicate a large increase in ethanol intake and preference even though the rats had access to ethanol at all times in their home cages. Moreover, the data in Figs. 2 and 3 indicate that 10 min of daily stimulation is far more effective than 30 min in terms of increasing ethanol intake, $F(1,22)=48.00$, $p<0.001$, and preference, $F(1,22)=33.15$, $p<0.001$, over the 23 days of stimulation. The greater ethanol intake of the 10 min group was particularly evident during the first two weeks of stimulation. After this, the between groups differences declined markedly. This is reflected in the significant interaction between the main effect (% preference) and time, $F(1,22)=22.91$, $p<0.01$. Because the numbers of drinkers in the 5 and 20 min groups were so small (1 and 2, respectively), further statistical comparisons among groups were not made.

The pooled data for all 9 drinkers, compared to the 11 non-drinkers which are presented in Figs. 4 and 5 also show large differences in terms of ethanol intake, $F(1,22)=312.63$, $p<0.001$, and preference, $F(1,22)=388.50$, $p<0.001$, over the 23 days of stimulation.

The time course of the development of ethanol drinking in the two groups was very different. The non-drinkers showed a progressive, linear increase over the duration of the experiment. In contrast, the drinkers (particularly those in the 10 min group) showed an abrupt increase in ethanol intake and preference with the beginning of stimulation. The enhance-

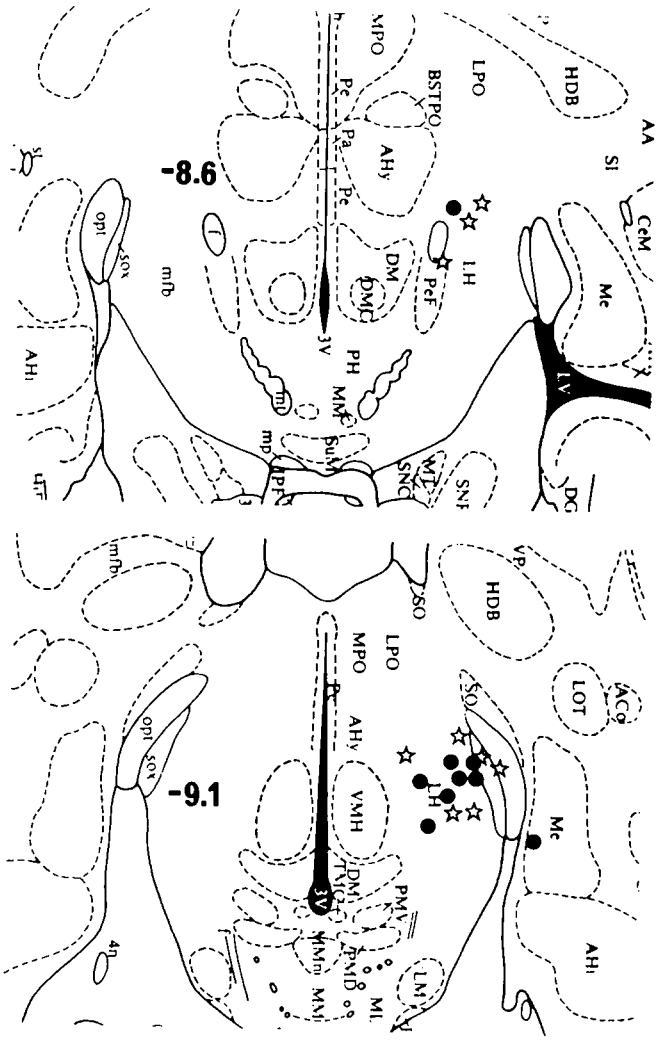

FIG. 1. Electrode locations presented on horizontal schematics from the atlas of Paxinos and Watson [9]. The electrode sites at which the stimulation produced post stimulation ethanol drinking are indicated as stars whereas the sites at which the stimulation did not alter ethanol intake are indicated as solid circles. One ineffective electrode penetrated the base of the brain and another was located in the thalamus.

ment became essentially asymptotic after about one week, although, as the data in Figs. 2 and 3 show, the 30 min group reached their asymptote rather later.

Whereas the total food intake and body weight of the drinkers was nearly identical to that of the non-drinkers, the drinkers had an important supplementary source of energy, the ethanol they drank. In terms of total energy intake per g of body weight the drinkers significantly exceeded non-drinkers, 1.09 vs. 0.96 Kj, $F(1,22)=21.12$, $p<0.001$.

The effect of changing the food source from powder to pellets was both dramatic and unexpected. Although a substantial reduction in ethanol intake and preference also occurred in the control group, the inhibitory effect on the drinkers was much greater. The dietary change reduced the ethanol consumption of the drinkers from nearly 6 g/kg/day to barely 1 g/kg/day. At this point the ethanol intake of the drinkers and non-drinkers was nearly identical. Further, it was nearly identical to their baseline consumption over 3

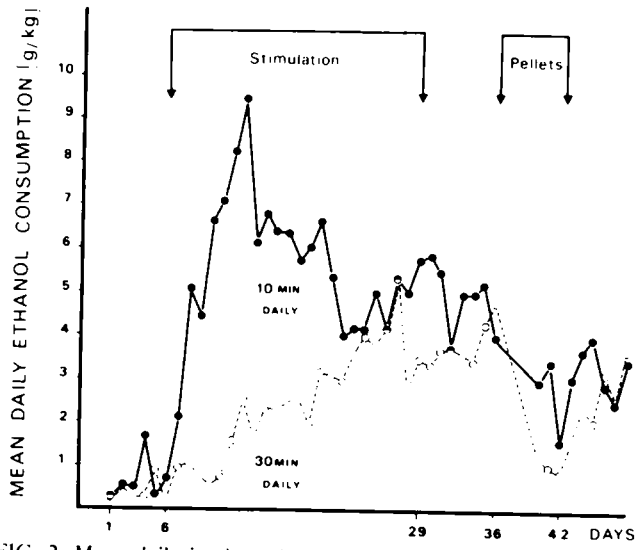

FIG. 2. Mean daily intake (g/kg) of continuously available ethanol produced by low level electrical stimulation of the lateral hypothalamus. The treatment of the two groups (N = 3 in each group) differed only in the duration of the daily stimulation session (10 min or 30 min). Over the 23 days of stimulation the ethanol intake of the 10 min group was significantly ($p<0.001$) greater than that of the 30 min group. The rats had ad lib access to powdered lab chow in their home cages except during the indicated 6 day period when the same chow was presented in pellet form.

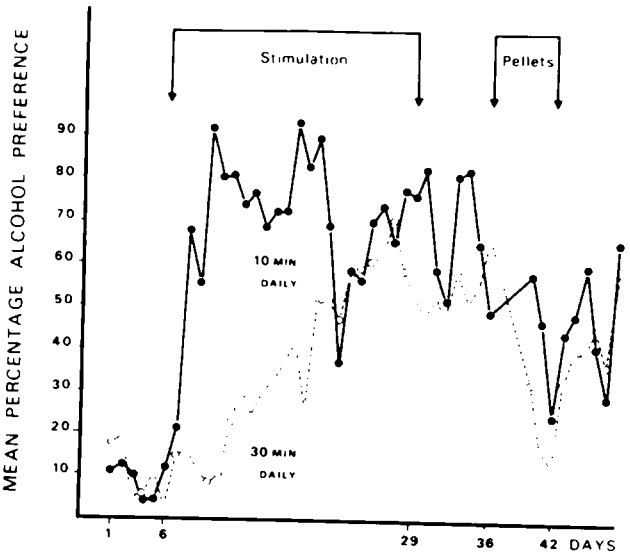

FIG. 3. Mean preference for continuously available ethanol expressed as a percentage of total daily fluid intake produced by low level electrical stimulation of the lateral hypothalamus. The treatment of the two groups (N=3 in each group) differed only in the duration of the daily stimulation session (10 min or 30 min). Over the 23 days of stimulation the ethanol preference of the 10 min group was significantly ($p<0.001$) greater than that of the 30 min group. The rats had ad lib access to powdered lab chow in their home cages except during the indicated 6 day period when the same chow was presented in pellet form.

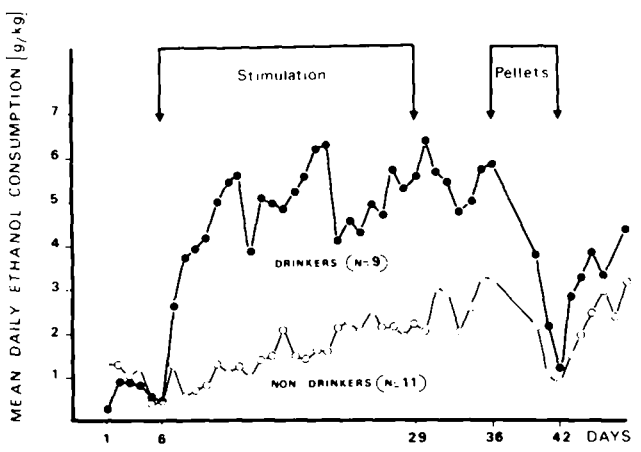

FIG. 4. Mean daily intake (g/kg) of continuously available ethanol produced by low level electrical stimulation of the lateral hypothalamus. Over the 23 days of stimulation the ethanol intake of the drinkers (N=9) was significantly ($p<0.001$) greater than that of the non-drinkers (N=11). The rats had ad lib access to lab chow in their home cages except during the indicated 6 day period where the same chow was presented in pellet form.

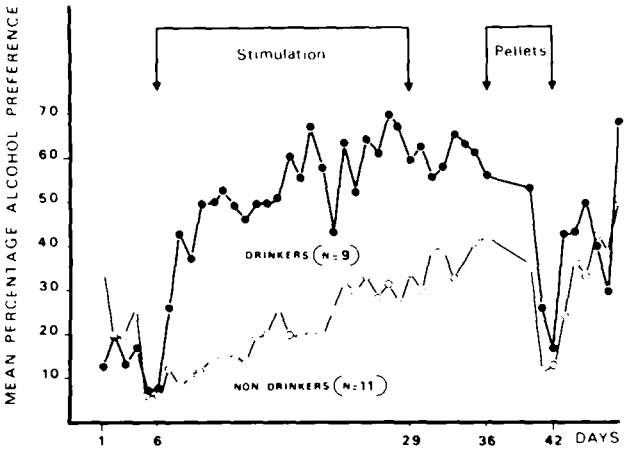

FIG. 5. Mean preference for continously available ethanol expressed as a percentage of total daily fluid intake produced by low level electrical stimulation of the lateral hypothalamus. Over the 23 days of stimulation the ethanol preference of the drinkers (N=9) was significantly ($p<0.001$) greater than that of the non-drinkers (N=11). The rats had ad lib access to lab chow in their home cages except during the indicated 6 day period where the same chow was presented in pellet form.

TABLE 1

BODY WEIGHT EXPRESSED AS A PERCENTAGE OF PRE-STIMULATION WEIGHT

	Day 29 (end of stimulation)	Day 36 (start of pellet diet)	Day 42 (end of pellet diet)	Day 48 (end of experiment)
Drinkers	114.9%	116.8%	127.6%	125.4%
Non drinkers	116.7%	121.9%	128.5%	128.1%

weeks earlier. The reinstatement of the powdered diet produced a rapid return towards their former levels of ethanol intake.

The change to the pellets produced rapid weight gains in both the drinkers and non-drinkers. On their return to the original powdered diet the rats' weight remained unchanged at the higher level, whereas the ethanol drinking rapidly increased towards their previous levels.

DISCUSSION

The present data show that electrical stimulation of the lateral hypothalamus can produce a large magnitude, rapid onset and long lasting increase in both ethanol intake and preference. This enhancement occurs even though the ethanol is available at all times in the home cage. Thus, it can be completely dissociated from the alternate day availability effect which is common to most studies in this area [1–5, 11, 13].

These data further show that the 30–60 min sessions used in all of the experiments to date [1–5, 8, 11–13] are far too long to produce a maximal enhancement effect. In agreement with Wayner and Greenberg [13] the present experiment shows that 30 min of daily stimulation by itself produces only a rather small enhancement of ethanol drinking (approximately 1.5 g/kg/day). However, decreasing the stimulation session length to 10 min increases the enhancement to about 4.5 g/kg/day. With one third the stimulation, the enhancement is three times as great. The superiority of the short stimulation sessions is particularly apparent during the first two weeks of stimulation.

Within the above limits of stimulation train length, one can relate the ethanol enhancement seen in the various studies to total effective daily stimulation charge (frequency × pulse width × pulse amplitude × stimulation time). These calculations indicate that the rats in the 10 min group received less than 5% of the total stimulation charge used in any of the previous studies reporting an enhancement effect. More brain stimulation clearly produces less of an effect. These data do not permit the specification of the optimal stimulation charge. They merely show that it is far lower than any used to date.

The striking effects of stimulation session length permit some speculation as to the causes of the post stimulation increase in ethanol consumption. An obvious explanation is that it is a response to the general stress produced by non-contingent brain stimulation. The ethanol drinking would be reinforced by its anxiolytic or stress reducing properties. However, the present data raise serious difficulties for this

explanation since it would require that 30 min of stimulation be less stressful than 10 min of stimulation. For more of a stressor to be less stressful is both counterintuitive and contraindicated by a large body of stress research [10]. The stress explanation is also contraindicated by the report that following ventromedial hypothalamic stimulation which should be at least as stressful as lateral hypothalamic stimulation, there appears to be an inhibition of ethanol intake [11].

The high energy requirement of the drinkers indicates that this paradigm produces a large and significant alteration in energy balance. However, the present data do not allow the determination of the source of this altered energy balance. It could be due to energetically wasteful substrate cycling or to increased thermogenesis caused by the ethanol. Alternately or additionally it could represent increased thermogenesis produced by the drink-inducing hypothalamic stimulation. It has recently been shown that repeated self-stimulation testing produces increased thermogenesis [6].

Merely changing the diet from powder to pellets of the same composition produced a large reduction in ethanol intake and preference in every rat. This voluntary abstinence shows that even the heaviest drinkers (more than 9 g/kg/day) were not ethanol dependent. More important, however, the fact that the decreased ethanol consumption is associated with rapid weight gains suggests that the ethanol consumption may be modulated by the animals' state of energy balance. The positive energy balance reflected by the rapid weight gain may have caused a compensatory decrease in ethanol intake.

There is also the possibility that the rats would not have acquired the ethanol drinking in the first place if they had been started on a diet of pellets. None of the studies to date has specified whether powder or pellets were used to maintain the rats. However, since various forms of pellets are normally used when the quantification of intake is not important, it seems likely that in at least some of the earlier studies the enhancement was obtained on a pellet diet.

Overall these data suggest a novel explanation for the acquisition of ethanol drinking produced by lateral hypothalamic stimulation. They suggest that it is a response to the negative energy balance induced by the stimulation [6,7]. Because ethanol is a source of rapidly metabolizable energy [15] its ingestion would serve to quickly reduce the negative energy balance induced by the brain stimulation. Any anxiolytic or stress reducing properties of ethanol would be an additional source of reinforcement. If the energetically wasteful neuroendocrine-metabolic cycle produced by the hypothalamic stimulation [6,7] became conditioned to the test situation it would tend to persist even after the stimulation was discontinued. According to this view, the reduction in ethanol intake produced by the dietary change and the subsequent increase following the reinstatement of the original diet, would be seen as alterations in a conditioned response produced by altering a major environmental cue.

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