ELSEVIER

European Journal of Pharmacology 357 (1998) 109–113

ejp

# Stimulation of locomotor activity by voluntarily consumed ethanol in Sardinian alcohol-preferring rats

Giancarlo Colombo [a,*], Roberta Agabio [b], Carla Lobina [b], Roberta Reali [b], Giovanni Vacca [b], Gian Luigi Gessa [b]

[a] *C.N.R. Center for Neuropharmacology, c/o 'Bernard B. Brodie' Department of Neuroscience, University of Cagliari, Via Porcell 4, I-09124 Cagliari, Italy*

[b] *'Bernard B. Brodie' Department of Neuroscience, University of Cagliari, Italy*

Received 5 March 1998; revised 24 July 1998; accepted 28 July 1998

**Abstract**

Stimulation of motor activity induced by ethanol has been proposed to reflect the positive reinforcing properties of the drug. The present study was designed to assess whether voluntary ethanol intake would stimulate locomotor activity in Sardinian alcohol-preferring (sP) rats, selectively bred for high ethanol preference and consumption. Rats were habituated to a) consume either water alone (water-consuming rats) or ethanol (10%, v/v) as free choice together with water (ethanol-consuming rats) according to a 15-min limited access protocol for 10 consecutive days prior to the test, and b) explore an open field for 10 min immediately after the drinking session in a trial on 3 consecutive days before the test. On the test day, voluntary ethanol consumption in ethanol-consuming rats averaged 1.2 g/kg. Values for activity measures (time spent moving, number of square crossings and number of rearings) were significantly higher in ethanol- than in water-consuming rats at both 5- and 10-min intervals. These results suggest that the euphorigenic effects of ethanol, supposedly represented by the stimulation of locomotor activity, are part of the reinforcing properties of ethanol in sP rats. 

*Keywords:* Sardinian alcohol-preferring (sP) rat; Ethanol voluntary intake; Locomotion; Positive reinforcing property; Dopamine mesolimbic system; Open field test

## 1. Introduction

Several lines of evidence indicate that the positive reinforcing and locomotor-stimulating effects of drugs of abuse are homologous phenomena, in that they result from activation of common biological mechanisms (see Wise and Bozarth, 1987). Indeed, both reinforcement and locomotor stimulation elicited by several addicting drugs, including amphetamine, cocaine, opiates, phencyclidine, cannabis and nicotine, have been reported to involve the activation of the mesolimbic dopamine system (see Wise and Bozarth, 1987).

As with the other substances of abuse, low to moderate doses of ethanol have been extensively reported to (a) increase the firing rate of ventral tegmental dopaminergic neurons (Gessa et al., 1985; Brodie et al., 1990) and, in turn, dopamine release in the nucleus accumbens in rats (e.g., Imperato and di Chiara, 1986; Wozniak et al., 1991; Yoshimoto et al., 1991; Blanchard et al., 1993; Heidbreder and de Witte, 1993; Weiss et al., 1993), and (b) stimulate spontaneous locomotor activity in rodents (e.g., Read et al., 1960; Carlsson et al., 1972; Imperato and di Chiara, 1986; Waller et al., 1986; Moore et al., 1993; Päivärinta and Korpi, 1993).

Ethanol intake in selectively bred ethanol-preferring rats is sustained by the positive reinforcing properties of the drug (see Crabbe and Li, 1995). Thus, if ethanol-induced stimulation of locomotor activity is an expression of the positive reinforcing properties of ethanol (see Wise and Bozarth, 1987), voluntary ethanol intake in these rat lines should indeed stimulate locomotor activity. This hypothesis was tested in the present study by assessing, in an open field test (see Kelley, 1993), the effect of voluntarily

* Corresponding author. Tel.: +39-70-675-8432; Fax: +39-70-657237; E-mail: colomb@unica.it

0014-2999/98/$19.00 
PII: S0014-2999(98)00560-3

Table 1
Parameters measured on the test day in ethanol-consuming ($n = 7$) and water-consuming ($n = 8$) Sardinian alcohol-preferring (sP) rats

| | Ethanol-consuming sP rats | Water-consuming sP rats |
|---|---|---|
| Ethanol intake (g/kg) | 1.2 ± 0.1 (0.9–1.7) | – |
| Ethanol solution intake (ml/kg) | 15.0 ± 1.5 (11.3–20.8) | – |
| Water intake (ml/kg) | 0.5 ± 0.1 (0.0–1.3) | 0.2 ± 0.1 (0.0–0.7) |
| Total fluid intake (ml/kg) | 15.5 ± 1.4[a] (11.8–21.0) | 0.2 ± 0.1 (0.0–0.7) |
| Preference ratio (%) | 96.2 ± 1.1 (90.9–100) | – |
| Blood ethanol levels (mg%) | 45.5 ± 9.6 (15.8–87.7) | – |

Ethanol, water and total fluid intake, as well as the preference ratio (defined as percent of ethanol solution consumed over total fluid intake) were recorded at the end of the 15-min drinking session preceding the trial in the open field. Blood samples were collected immediately after the end of the trial in the open field from the tip of the tail of alcohol-consuming rats; blood ethanol levels were measured by gas chromatography.
Each value is the mean ± S.E.M. for $n$ rats. The value range is indicated in parentheses.
[a] $P < 0.001$ with respect to water-consuming rats (unpaired, two-tailed Mann–Whitney test).

consumed ethanol on spontaneous locomotor activity in Sardinian alcohol-preferring (sP) rats, one of the five rat lines selectively bred worldwide for high preference and consumption of ethanol over water (see Colombo, 1997).

## 2. Materials and methods

### 2.1. Animals

Fifteen male sP rats, from the 39th generation and approximately 6 months old, were used. Rats were individually housed in standard plastic cages with wood-chip bedding. The animal facility was under a reversed 12:12 h light–dark cycle (lights on at 2100), at a constant temperature of 22 ± 2°C and relative humidity of 60%. The rats were habituated to handling.

### 2.2. Open field apparatus

Rat locomotor activity was tested with an open-field apparatus, consisting of a square box (60 × 60 × 35 ($h$) cm) of opaque, black Plexiglas. The floor was divided into 9 equal squares by thin white lines. One of the walls was made of transparent Plexiglas. The apparatus was located in a quiet dimly lit room, adjacent to the housing room. Rat behavior was monitored by three observers placed behind a screen 2 m from the apparatus and unaware of the rat allocation.

### 2.3. Procedure of locomotor activity testing

The rats were divided into two groups: ethanol-consuming ($n = 7$; body weight: 535.3 ± 17.3 g) and water-consuming ($n = 8$; body weight: 495.4 ± 13.9) rats. Ethanol-consuming rats were offered two bottles containing ethanol (10% v/v, in tap water) and tap water, respectively, in daily 15-min drinking sessions (between 0900 and 1300). Two bottles containing tap water were presented to water-consuming rats during the drinking session. Between sessions, a single bottle containing water was available to each rat group. Ethanol and water intakes were monitored in both groups by weighing the two bottles (0.1 g accuracy) at the end of the drinking session. Bottles were refilled every day with fresh solutions and their positions interchanged at random to avoid development of position preference. After one week of habituation, all rats showed stable ethanol and water intakes during the daily drinking sessions.

On the subsequent days, rats underwent the trials in the open field. Trials were conducted immediately after the end of the drinking session for four consecutive days. Locomotor activity was recorded only at the last trial (test day), the other three trials being intended for rat familiar-

Table 2
Results of the two-way analyses of variance for time spent moving, number of square crossings and number of rearings by ethanol-consuming ($n = 7$) and water-consuming ($n = 8$) Sardinian alcohol-preferring (sP) rats tested in the open field for 10 min immediately after the 15-min drinking session

| Source | Effect | $df$ | $F$ | $P$-level |
|---|---|---|---|---|
| Time spent moving | rat group | 1 | 16.1151 | 0.001472 |
| | time interval | 1 | 122.491 | < 0.000001 |
| | rat group × time interval | 1 | 0.7188 | 0.411867 |
| Number of square crossings | rat group | 1 | 27.9383 | 0.000147 |
| | time interval | 1 | 104.7225 | < 0.000001 |
| | rat group × time interval | 1 | 0.7188 | 0.024287 |
| Number of rearings | rat group | 1 | 8.9601 | 0.010371 |
| | time interval | 1 | 31.69298 | 0.000082 |
| | rat group × time interval | 1 | 0.65756 | 0.432017 |

ization with the test procedure. Trials lasted 10 min. In each trial, the rat was placed in the open field (namely, in the central square of the apparatus, facing away from the transparent wall) immediately after the end of the drinking session. At the end of the trial, the rat was replaced directly into its home cage and the open field was cleaned thoroughly. Rats from the two groups were tested alternatively.

### 2.4. Estimation of blood alcohol levels

On the test day, immediately after the end of the trial in the open field, a blood sample (20 μl) was collected from the tip of the tail of each ethanol-consuming rat for assessment of blood ethanol levels. Blood samples were diluted tenfold in distilled water containing 0.25 mg/ml propanol as internal standard. Samples were stored at 4°C, centrifuged at 3500 rpm for 5 min and immediately injected (1 μl injection volume) into a gas-chromatograph (Model F30, Perkin-Elmer, Beaconsfield, UK) equipped with flame ionization detector and glass column (1-m-long and 1/4-in inside diameter, packed with 5% Carbowax 20 M on 80–100 Mesh Chromosorb W). Temperatures of injector, column and detector were 150, 75 and 100°C, respectively. Nitrogen was used as the carrier gas (50 ml/min flow rate).

### 2.5. Data analyses

On the test day, the motor activity of each rat was evaluated during two 5-min intervals by recording (a) time (s) spent moving (including locomotion and rearings), (b) number of square crossings (measure of horizontal activity, scored any time the rat entered a square with at least one paw) and (c) number of rearings (measure of vertical activity, defined as the rat rising on its hindlegs with forelegs not touching the floor). Data for activity time, square crossings and rearing of ethanol- and water-consuming rats were expressed as mean ± S.E.M. and analysed by two-way (rat group; time interval) analysis of variance (ANOVA) with repeated measures on time intervals, followed by the Newman–Keuls test for post-hoc comparison.

Data for water and total fluid intakes of ethanol- and water-consuming rats were compared by means of the unpaired, two-tailed Mann–Whitney test.

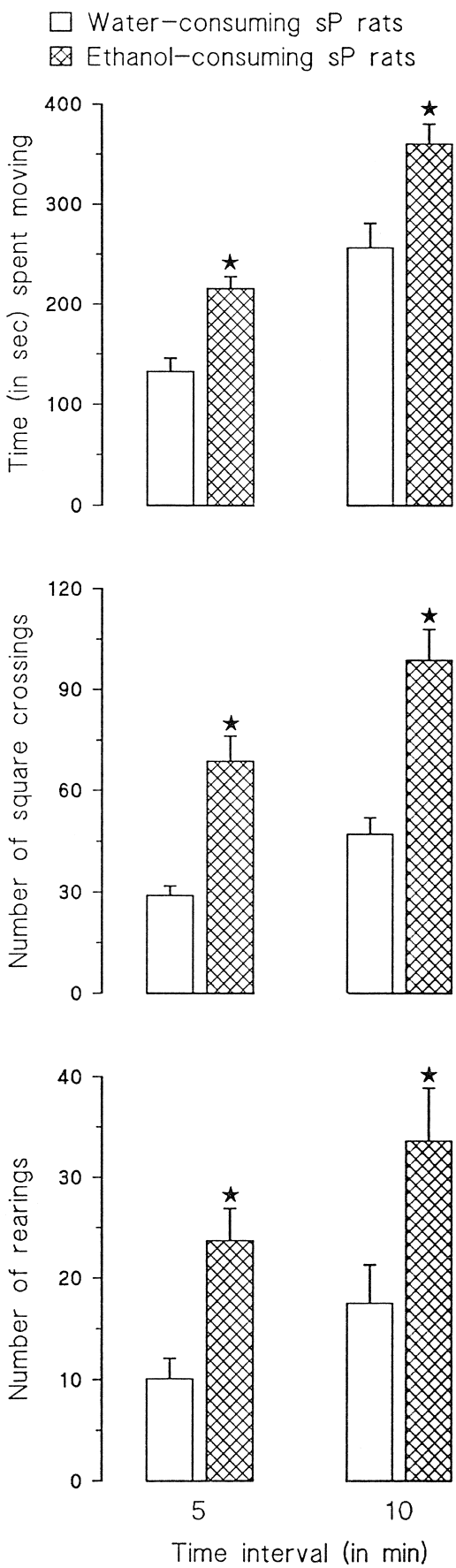


Fig. 1. Time spent moving (top panel), number of square crossings (center panel) and number of rearings (bottom panel) during a 10-min test in the open field in water-consuming ($n = 8$) and ethanol-consuming ($n = 7$) Sardinian alcohol-preferring (sP) rats during the first 5 min (5-min interval) and the entire test (10-min interval). Ethanol (10%, v/v) and water intakes occurred in the two-bottle, free choice protocol with water for 15 min immediately prior to the start of the open field trial. Each column represents the mean ± S.E.M. for $n$ rats. $* P < 0.001$ with respect to water-consuming rats (Newman–Keuls test).

## 3. Results

On the test day, the mean ethanol intake and preference ratios of ethanol-consuming rats during the 15-min drinking session were 1.2 g/kg and 96.2%, respectively (Table 1). Ethanol intake produced blood ethanol levels averaging 45.5 mg% at the end of the locomotor activity test. In water-consuming rats, the intake of water (the sole fluid available) during the 15-min drinking session averaged 0.2 ml/kg, more than 30-fold less than the total fluid intake of the ethanol-consuming rats.

When tested in the open field, ethanol-consuming rats were significantly more active than water-consuming rats at both time intervals (see Table 2 for ANOVA results). Maximal activation was recorded during the first 5-min interval, when ethanol-consuming rats spent approximately 65% more time moving, made approximately 135% more square crossings, and scored approximately 135% more rearings than did water-consuming rats (Fig. 1). At the end of the test (10-min interval), time spent moving, number of square crossings and number of rearings were approximately 40, 110 and 90%, respectively, higher in ethanol- than in water-consuming rats (Fig. 1).

## 4. Discussion

On the test day, (a) voluntary ethanol intake of ethanol-consuming sP rats during the 15-min drinking session was about 30-fold higher than the water intake of water-consuming sP rats and (b) the ensuing blood ethanol levels (averaging 45.5 mg%) were pharmacologically relevant, suggesting that intake was motivated by the search for pharmacological effects of ethanol and not just to satisfy fluid requirements.

The results of the open field test, performed immediately after the drinking session, indicated that ethanol-consuming rats were significantly more active than water-consuming rats, suggesting that voluntary ethanol intake had a motor stimulating effect. The blood ethanol levels, as monitored at the end of the activity test, were consistently within the range previously reported to stimulate locomotor activity after parenteral administration of ethanol (Gill et al., 1986). Maximal activation occurred during the first 5 min of the open field test, in agreement with previous reports demonstrating the short duration of this effect (Moore et al., 1993; Päivärinta and Korpi, 1993).

The results of the activity test also suggested that the euphorigenic effects of ethanol, as reflected by the stimulation of locomotor activity (see Wise and Bozarth, 1987), are part of the pharmacological effects of ethanol that sustain voluntary ethanol intake in sP rats. Furthermore, these results are in close agreement with those of Päivärinta and Korpi (1993), indicating that locomotor activity in alcohol-accepting AA rats was significantly increased after the voluntary intake of doses of ethanol averaging 0.9 g/kg in a 10-min drinking session. Thus, stimulation of locomotor activity by voluntarily consumed ethanol may be a common feature of rat lines genetically selected for high ethanol preference and consumption.

The likely neurochemical basis of this effect is activation of the dopamine mesolimbic system. Indeed, locomotor stimulation and reinforcement from drugs of abuse have been proposed to be homologous because both phenomena involve activation of the dopamine mesolimbic system (see Wise and Bozarth, 1987). Consistently, voluntary ethanol intake in a limited access protocol similar to that applied in the present study has been reported to stimulate dopamine turnover in mesolimbic areas of sP rats (Fadda et al., 1989).

In summary, the results of the present study (a) indicate that voluntary ethanol intake in ethanol-preferring sP rats, under a 15-min access period, is followed by an increase in motor activity, and (b) strengthen the hypothesis that stimulation of motor activity is an expression of the positive reinforcing properties of ethanol.

## Acknowledgements

The authors are grateful to Mrs. Marinella Boi and Mrs. M. Elena Vincis for animal care and breeding and to Mr. Hugh Sugden for language editing of the manuscript. The present study was partially supported by C.N.R. grant #91.04142.ST75 and grant #4173/11668 from Assessorato Igiene e Sanità, Regione Autonoma della Sardegna.

## References

Blanchard, B.A., Steindorf, S., Wang, S., Glick, S.D., 1993. Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. Alcoholism: Clin. Exp. Res. 17, 968–973.

Brodie, M.S., Shefner, S.A., Dunwiddie, T.V., 1990. Ethanol increases the firing rate of dopamine neurons of the rats ventral tegmental area in vitro. Brain Res. 508, 65–69.

Carlsson, A., Engel, J., Svensson, T.H., 1972. Inhibition of ethanol-induced excitation in mice and rats by a α-methyl-*p*-tyrosine. Psychopharmacologia (Berlin) 26, 307–312.

Colombo, G., 1997. Ethanol drinking behaviour in Sardinian alcohol-preferring rats. Alcohol Alcohol. 32, 443–453.

Crabbe, J.C., Li, T.-K., 1995. Genetic strategies in preclinical substance abuse research. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: the Fourth Generation of Progress. Raven Press, New York, NY, pp. 799–811.

Fadda, F., Mosca, E., Colombo, G., Gessa, G.L., 1989. Effect of spontaneous ingestion of ethanol on brain dopamine metabolism. Life Sci. 44, 281–287.

Gessa, G.L., Muntoni, F., Collu, M., Vargiu, L., Mereu, G., 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res. 348, 201–203.

Gill, K., France, C., Amit, Z., 1986. Voluntary ethanol consumption in

rats: an examination of blood/brain ethanol levels and behavior. Alcoholism: Clin. Exp. Res. 10, 457–462.

Heidbreder, C., de Witte, P., 1993. Ethanol differentially affects extracellular monoamines and GABA in the nucleus accumbens. Pharmacol. Biochem. Behav. 46, 477–481.

Imperato, A., di Chiara, G., 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 239, 219–228.

Kelley, A.E., 1993. Locomotor activity and exploration. In: Sahgal, A. (Ed.), Behavioural Neuroscience: a Practical Approach, Vol. 2. IRL Press, Oxford, pp. 1–21.

Moore, T.O., June, H.L., Lewis, M.J., 1993. Ethanol-induced stimulation and depression on measures of locomotor activity: effects of basal activity levels in rats. Alcohol 10, 537–540.

Päivärinta, P., Korpi, E.R., 1993. Voluntary ethanol drinking increases locomotor activity in alcohol-preferring AA rats. Pharmacol. Biochem. Behav. 44, 127–132.

Read, G.W., Cutting, W., Furst, A., 1960. Comparison of excited phases after sedatives and tranquilizers. Psychopharmacologia (Berlin) 1, 346–350.

Waller, M.B., Murphy, J.M., McBride, W.J., Lumeng, L., Li, T.-K., 1986. Effect of low dose ethanol on spontaneous motor activity in alcohol-preferring and -nonpreferring lines of rats. Pharmacol. Biochem. Behav. 24, 617–623.

Weiss, F., Lorang, M.T., Bloom, F.E., Koob, G.F., 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267, 250–258.

Wise, R.A., Bozarth, M.A., 1987. A psychomotor stimulant theory of addiction. Psychol. Rev. 94, 469–492.

Wozniak, K.M., Pert, A., Mele, A., Linnoila, M., 1991. Focal application of alcohols elevates extracellular dopamine in rats brain: a microdialysis study. Brain Res. 540, 31–40.

Yoshimoto, K., McBride, W.J., Lumeng, L., Li, T.-K., 1991. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 9, 17–22.