Pharmacology Biochemistry and Behavior, Vol. 2, pp. 847–850. ANKHO International Inc., 1974. Printed in the U.S.A.

BRIEF COMMUNICATION

Behavioral Stimulation Induced by Ethanol Withdrawal

SVEN AHLENIUS AND JÖRGEN ENGEL

Department of Pharmacology, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden

(Received 10 February 1974)

AHLENIUS, S. AND J. ENGEL. *Behavioral stimulation induced by ethanol withdrawal.* PHARMAC. BIOCHEM. BEHAV. 2(6) 847–850, 1974. – A model for the study of an ethanol withdrawal syndrome on operant behavior is described. Rats maintained on 16% w/v solutions of ethanol for several months were trained to perform on a DRL-15 schedule. On withdrawal of ethanol the interresponse times were significantly shortened concomitant with an increase in the total number of responses.

Ethanol Operant behavior Withdrawal

VARIOUS experimental models have been used in the study of ethanol dependence and ethanol withdrawal [7,11]. Mostly, the withdrawal syndrome obtained with these models has been rather severe, including convulsions and occasionally death. Recently, Cicero *et al.* [6] have described a new method in which the animals have been exposed to ethanol for a long period starting at 21 days of age. With this procedure "upon withdrawal of alcohol the rats were found to be extremely hyperactive and appeared to be engaged in frantic, highly disorganized, exploratory behavior in an open field."

In the present investigation using a similar procedure the effects upon withdrawal of ethanol have been studied in rats trained to perform a food-reinforced lever-pressing behavior, maintained by a DRL (differential reinforcement of low rate)-schedule, which engenders very low rates of responding. A behavior thus maintained is known to be sensitive to drug-induced stimulation [9].

METHOD

Animals

Pregnant rats of the Sprague-Dawley strain (Anticimex, Stockholm) were used. All animals were born in the department and the birth was noted within 12 hr. At the age of 16 days the animals in four litters were weaned. The rats were then exposed to one of three different ethanol solutions, 4, 8, or 16%, or given water ad lib. Only the animals subjected to the 4 and 8% ethanol solutions displayed a growth curve comparable to that of the controls (Fig. 1). At two months of age four male rats of the animals given 8% ethanol were chosen for the behavioral experiment and maintained on 16% ethanol, since a continuation on the 8% solution does not induce any behavioral changes upon withdrawal (unpublished data).

Behavioral Procedure

After two months on 16% ethanol the four male rats were food-deprived and kept individually at a constant weight. The rats were trained to lever press on a food pellet (Noyes 45 mg) reinforced DRL-15 Schedule (Differential Reinforcement of Low Rate) in standard behavioral chambers (Model E 3125A, Grason-Stadler). On this schedule, depression of the lever produced a food pellet only if the response followed the preceding lever depression by at least 15 sec. A premature response (less than 15 sec after the last response) reset a clock so that the 15 sec interval began again.

The inter-response times (IRT, interval between successive responses) were divided in 3 sec categories: 0–3, 3–6 etc. Presses spaced more than 30 sec apart were collected in a last category. Lever-press responses were recorded on digital counters and categorized automatically. For each session a mean IRT was calculated. A grand mean for the control (n = 11) and the different treatments was calculated and a 98% confidence interval determined for the differences between the means [12].

Each rat was exposed to sessions 5 days a week. The rats were trained in a two hour session until they reached a stable baseline of lever-pressing. Experimental sessions consisted of a 15 min adaptation period, immediately followed by a 60 min period, in which responses were recorded.

Ethanol was withdrawn immediately after a control session and replaced by water. After three daily sessions on

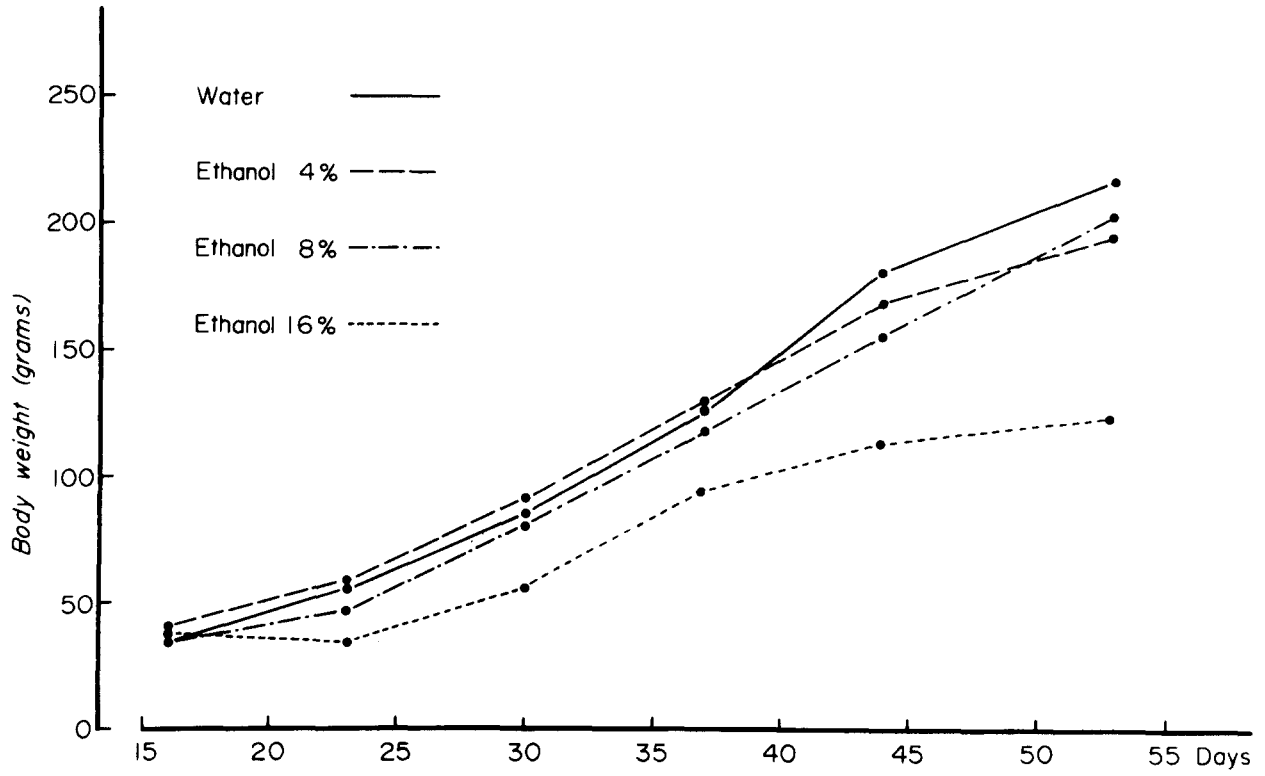

FIG. 1. Weight curves for rats kept at various concentrations of ethanol from Day 16. Ethanol was given orally in four different concentrations w/v (for further details see METHOD). Shown are the means of 6–8 animals.

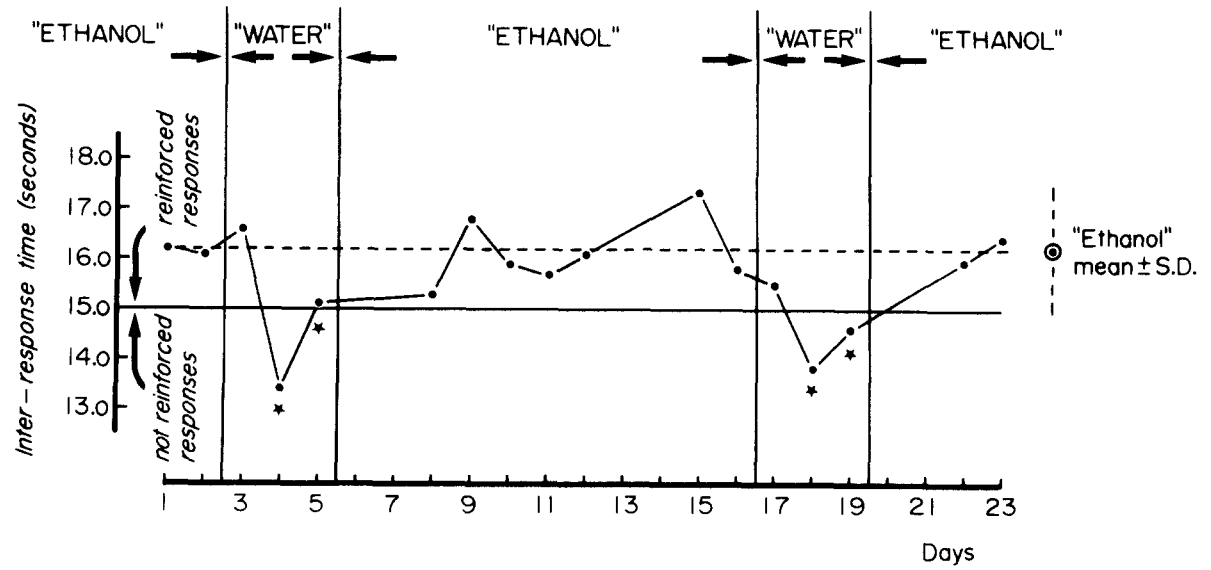

FIG. 2. Effects of ethanol (16% w/v orally) withdrawal on food-reinforced leverpressing behavior in rats, maintained on a DRL-15 schedule. Shown are the mean IRTs of four rats. The broken line represents the grand mean IRT for the ethanol control sessions (n = 11). ★ $p<0.01$ (difference between grand mean IRT for ethanol controls and the mean from the water sessions compared with zero [12].

water, ethanol was reintroduced immediately after the third session. This procedure was replicated once on the same animals. For further details see Fig. 2.

RESULTS AND DISCUSSION

Rats exposed to ethanol displayed a frequency distribution of the lever-presses very similar to that obtained in untreated animals [2,3]. On the first day after ethanol withdrawal there were no obvious changes in the lever-pressing behavior of the animals. However, on the second and third day on water, the IRT distributions showed a statistically significant increase in the frequency of short IRTs (Fig. 2 and 3). This effect was replicated once (see Fig. 2). When ethanol was reintroduced the animals returned to the ethanol control level of lever-pressing. The significant decrease in the IRTs under water on Day 2 and Day 3 was accompanied by a significant increase in the total number of responses emitted (Table 1).

It should be noted that in spite of the fact that the shortening of IRTs during ethanol withdrawal resulted in smaller amounts of reinforcements being received, the rats persisted in pressing the lever even at an enhanced rate. This stimulation on DRL has previously been shown in rats treated with the central stimulant amphetamine [3,14]. In man, different signs of overactivity are seen during ethanol withdrawal [13]. The central stimulating effects of amphetamine in animals and man are considered to be mediated via a release of central catecholamines [4,8]. Whether similar mechanisms are involved in the behavioral stimulation observed during ethanol withdrawal remains to be clarified. The stimulatory effects on behavior observed after acute ethanol administration in animals [5] and man [1] have been suggested to involve central catecholamines.

There was a marked increase in the amount of fluid intake on the first day on water (Table 1). On the ensuing two days on water there were no significant changes in the amount of fluid intake as compared to ethanol controls. It should be noted that the amount of fluid consumed under ethanol is in substantial agreement with the normal water intake reported for the rat [10].

After the behavioral experiments had been discontinued, a separate experiment was performed in which the animals were presented both ethanol and water. Under these circumstances it was found that the total fluid intake was markedly increased, up to 60 ml per 24 hr after one week. The ethanol intake constituted about 37% of this total amount. It is well known that adult rats do not voluntarily ingest ethanol in the concentrations used in the experiment. Thus the presentation of ethanol at an early age seems to be a prerequisite for a significant ingestion of ethanol in adult rats. Similar results have previously been reported by Cicero *et al.* [6], using about the same percent solutions of ethanol. Taken together this seems to be a reliable method for the induction of ethanol dependence and the study of ethanol withdrawal in the rat.

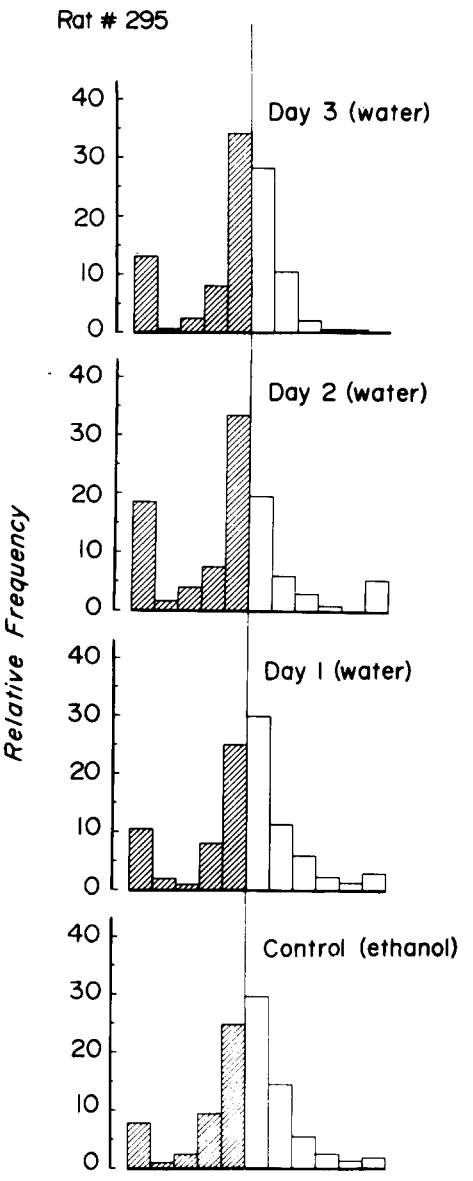

FIG. 3. Relative freuency distributions of time intervals between successive lever-pressing responses from one rat.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (B74-14X-2157-06). J. E. is supported by the Swedish Board for Technical Development. The skillful technical assistance of Mr. Kenn Johannessen and Mrs. Ingela Olsson is gratefully acknowledged.

TABLE 1

		Inter-Response Time (sec)	Total Responses	Fluid Consumption (ml/24 hr)	Weight (g)
Ethanol		16.2 ± 1.27*	182 ± 60	31.4 ± 2.19	293 ± 31
Water	Day 3	16.6 ± 2.44^{NS}	186 ± 57^{NS}	59.5 ± 10.63†	306 ± 27†
	4	13.4 ± 0.54†	258 ± 10†	29.0 ± 13.11^{NS}	298 ± 31^{NS}
	5	15.1 ± 2.21†	236 ± 34†	25.0 ± 8.16^{NS}	289 ± 29^{NS}
	17	15.5 ± 2.85^{NS}	218 ± 30^{NS}	65.0 ± 10.80†	305 ± 28†
	18	13.8 ± 2.73†	264 ± 55†	33.8 ± 12.50^{NS}	297 ± 30^{NS}
	19	14.6 ± 1.13†	245 ± 17†	not measured	288 ± 33^{NS}

*mean ± S.D. (n = 4) †$p<0.01$

REFERENCES

1. Ahlenius, S., A. Carlsson, J. Engel, T. Svensson and P. Södersten. Antagonism by alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man. *Clin. pharmac. Ther.* **14:** 586–591, 1973.
2. Ahlenius, S. and J. Engel. Effects of small doses of haloperidol on timing behaviour. *J. Pharm. Pharmac.* **23:** 301–302, 1971.
3. Ahlenius, S. and J. Engel. Effects of a dopamine (DA)-β-hydroxylase inhibitor on timing behaviour. *Psychopharmacologia* **24:** 243–246, 1972.
4. Carlsson, A. Amphetamine and brain catecholamines. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven Press, 1970, pp. 289–300.
5. Carlsson, A., J. Engel and T. Svensson. Inhibition of ethanol-induced excitation in mice and rats by α-methyl-p-tyrosine. *Psychopharmacologia* **26:** 307–312, 1972.
6. Cicero, T. J., S. R. Snider, V. J. Perez and L. W. Swanson. Physical dependence on and tolerance to alcohol in the rat. *Physiol. Behav.* **6:** 191–198, 1971.
7. Freund, G. Alcohol, barbiturate, and bromide withdrawal syndrome in mice. In: *Recent Advances in Studies of Alcoholism,* edited by N. K. Mello and J. H. Mendelson. Washington: H.S.M. Publication No. 71–9045, 1971, pp. 453–471.
8. Jönsson, L. -E., L. -M. Gunne and E. Änggård. Effects of α-methyl-tyrosine in amphetamine-dependent subjects. *Pharmac. Clin.* **2:** 27–29, 1969.
9. Kelleher, R. T. and W. H. Morse. Determinants of the specificity of behavioral effects of drugs. *Rev. Physiol.* **60:** 1–56, 1968.
10. Kutscher, C. L. Species differences in the interaction of feeding and drinking. *Ann. N.Y. Acad. Sci.* **157:** 539–552, 1969.
11. Ogata, H., F. Ogata, J. Mendelson and N. K. Mello. A comparison of techniques to induce alcohol dependence and tolerance in the mouse. In: *Recent Advances in Studies of Alcoholism,* edited by N. K. Mello and J. H. Mendelson. Washington: H.S.M. Publication. No. 71-9045, 1971, pp. 472–507.
12. Scheffé, H. *The Analysis of Variance.* New York: John Wiley and Sons, 1959, pp. 160–190.
13. Seixas, F. A. and S. Eggleston. Eds. Alcoholism and the central nervous system. *Ann. N.Y. Acad. Sci.* **215:** 1–389, 1973.
14. Sidman, M. Technique for assessing the effects of drugs on timing behavior. *Science* **122:** 925, 1955.