Pharmacology Biochemistry & Behavior, Vol. 11, pp. 581–584. Printed in the U.S.A.

Ethanol Withdrawal and Discriminative Motor Control: Effect of Chronic Intake Level[1]

MAISY TANG AND JOHN L. FALK

Department of Psychology, Busch Campus, Rutgers University, New Brunswick, NJ 08903

Received 20 August 1979

TANG, M. AND J.L. FALK. *Ethanol withdrawal and discriminative motor control: effect of chronic intake level.* PHARMAC. BIOCHEM. BEHAV. 11(5)581–584, 1979.—Rats drinking a low concentration (2.5%) of ethanol in a chronic, schedule-induced polydipsia situation failed to show withdrawal signs as measured by a discriminative motor control task. When the concentration was raised to 5% ethanol, withdrawal signs were evident, confirming a previous study.

Ethanol Motor control Withdrawal Schedule-induced polydipsia

IN PREVIOUS research from our laboratory, withdrawal from chronic schedule-induced ethanol overindulgence (daily intake averaged about 10–11 g ethanol/kg/day over several months) resulted in marked dyskinesia as measured by a discriminative motor control task [8]. This chronic ethanol dipsogenic regimen was shown earlier to produce severe physical dependence [4]. The present experiment was designed to determine if chronic ethanol intake at a somewhat lower level would produce withdrawal dyskinesia. Consequently, the same dipsogenic, intermittent-food schedule was used as in the above studies with the exception that a 2.5% ethanol solution was made available to the rats rather than the usual 5.0% ethanol solution. Our aim was to answer the question of a possible insidious development of physical dependence even if the daily ethanol pattern of ingestion remained within the metabolic capacity.

METHOD

Animals

Four male, albino, Holtzman rats with a mean body weight of 357 g (range: 315–407) were used in the present study.

Discriminative Motor Control

The chamber used to evaluate motor competence consisted of a Plexiglas chamber (25×30×30 cm) with stainless steel front and rear panels. A force-sensitive manipulandum and a food pellet receptacle were both mounted on the front panel with enough distance (17 cm) between them to prevent an animal from touching the manipulandum and reaching into the pellet tray simultaneously. The manipulandum was shielded so that an animal could only touch it with a single paw. This manipulandum rested on a force transducer unit (Statham Model UC3 strain gauge mounted to a Statham Model UL4 load cell) which was coupled to a bridge amplifier (Statham Model SC1105) that connected directly into a Lab-8 digital computer (Digital Equipment Corp.) The computer was programmed to deliver a 45 mg Noyes pellet into the food cup via a pellet dispenser (Gerbrands Model D-1) at the successful completion of the motor task: holding the manipulandum continuously within a force band of 15 to 30 g for 1.5 sec. Each motor performance session terminated after the delivery of 50 pellets. Motor performance was evaluated by four measures that were calculated from overall session values:

(A) In-band efficiency: $\frac{\text{minimum possible in-band time}}{\text{in-band time}}$;

(B) Tonic accuracy: $\frac{\text{in-band time}}{\text{total response time}}$;

(C) Work rate: $\frac{\text{total response time}}{\text{session time}}$;

and (D) Dyskinesia: total number of entrances into force band. The design of the apparatus [8] and the methodology involved [3,8] have been described previously in greater detail.

Procedure

Motor control training. During this phase of the experiment all animals were housed individually in standard stainless steel Acme cages in a temperature-controlled room with 12-on-12-off illumination condition. Water was continuously available in these home cages. Animals were placed on food deprivation for 24 hr before the start of the first training session. Thereafter, a food ration (Purina lab chow, pelleted) was given in the home cage immediately after each daily training session in order to maintain the animals at 80% of their free-feeding body weight. The animals were first shaped to hold the manipulandum for at least 0.5 sec within a wide force band limit (1 to 40 g). Over the next 4 weeks both the band width and the required hold time was sequentially changed toward the final criterion (1.5 sec within a 15–30 g band). After an additional two months, all animals had

[1] Supported by PHS grants DA1110 and AA00253.

[2] Send reprint requests to: Maisy Tang, Psychology Building, Busch Campus, Rutgers University, New Brunswick, NJ 08903.

TABLE 1
SCHEDULE-INDUCED CHRONIC ETHANOL INTAKES (LAST 10 DAYS) AND BLOOD ETHANOL CONCENTRATIONS

% EtOH in Drinking Fluid	Mean Daily EtOH Intake (g/kg)	Blood EtOH Concentration (mg/dl)
2.5	7.86 ± 0.60*	0200 hr: 9.5 (0–38)† 1400 hr: undetect.
5.0	9.93 ± 0.45	0200 hr: 128 (71–203) 1400 hr: 97 (77–129)

*S.E.
†Range

stabilized their motor performance. Each animal was then placed in a Plexiglas champer (27x30x24 cm) in a continuously illuminated room. Each chamber was equipped with a stainless-steel food pellet receptacle and water was continuously available from a stainless-steel, ball-bearing drinking spout (Ancare TD-300) which was attached to a 250 ml Nalgene graduated cylinder. A 45 mg Noyes pellet was delivered automatically into the food tray every 2 min for 1 hr (feeding session) and was followed by 3 hr when no food was delivered. These 1-hr feeding sessions were alternated continuously with 3-hr no-food periods for the duration of the experiment. Thus, within each 24-hr period the intermittent-feeding schedule consisted of 6 one-hr feeding periods each separated by 3 hr of no food.

At 10 a.m. each day (1 hr after the last feeding session), animals were weighed, their overnight fluid intake recorded and their drinking tubes refilled. At this time every other day, the animals were put into the motor performance apparatus for a 50-pellet session. Any food rations necessary to maintain the animals at 80% body weight were given after the animals returned to their home cages from the motor task session, or immediately after body weight determinations on days when motor performance was not evaluated.

Low ethanol intake. The animals were placed on the intermittent-feeding schedule for 2 weeks at which point a 2.5% ethanol solution (v/v) was substituted for water as the drinking fluid. After 1 month of chronic 2.5% ethanol drinking, withdrawal effects on motor performance were evaluated at 5 and 10 hr post-withdrawal. Withdrawal consisted of substituting water for ethanol as the drinking fluid; 2.5% ethanol was replaced immediately after the 10-hr post-withdrawal session. A second ethanol withdrawal was introduced after two additional months of drinking 2.5% ethanol. Again, water was substituted for ethanol, and motor performance was evaluated at 5, 10 and 28 hr post-withdrawal.

High ethanol intake. A 5.0% ethanol solution (v/v) was substituted for water as the drinking fluid 12 days after the last ethanol withdrawal. Due to the extra calories supplied by the 5.0% ethanol, animals were allowed a motor performance session only every third day in order to prevent excessive weight gain. Ethanol was withdrawn at the end of one month of chronic 5.0% ethanol drinking. Motor performance was evaluated at 5, 10 and 28 hr post-withdrawal.

Blood alcohol levels. Blood ethanol concentrations were determined in all animals when a stable daily intake of 2.5% ethanol was obtained and redetermined after the intake of 5.0% ethanol had stabilized.

On blood sampling days, a 50 μl tail blood sample was collected at 0200 and 1400 hr (2 hr after the previous feeding session). The samples obtained were immediately prepared for ethanol level determinations using a gas chromatographic method developed by Le Blanc [7].

RESULTS

Low Ethanol Intake

The first row of Table 1 shows the mean ethanol intake for the last 10 days of the 3 month period during which the animals drank 2.5% ethanol in the chronic schedule-induction situation. The first row also shows that blood ethanol concentrations were quite low at this ethanol intake level. The effects of ethanol withdrawal on the motor performance measures are shown in Figures 1–4. Panels A and B of these figures present the results of withdrawal from the low (2.5% concentration) level of ethanol intake after 1 and 3 months of drinking. Repeated measure analysis of variance design (10, p. 105 ff) was used to evaluate the in-band efficiency, tonic accuracy, and work rate measures. The Friedman nonparametric analysis of variance was used in the case of the dyskinesia measure [9]. Within the time periods sampled, neither withdrawal at the 1 nor at the 3 month period resulted in significant disruption of motor performance on any of the measures.

High Ethanol Intake

The second row of Table 1 reveals that a greater daily ethanol intake occurred when the higher concentration (5.0%) ethanol solution was substituted for the 2.5% ethanol solution. The increased ethanol intake also is reflected in greater blood ethanol levels. Panel C of Figures 1–4 shows that withdrawal after 1 month of drinking the higher concentration resulted in disruption of motor performance. Overall analyses of variance of three of the measures are significant: in-band efficiency, $F(3,9)=7.16$, $p<0.01$, work rate, $F(3,9)=3.95$, $p<0.05$, and dyskinesia, $\chi^2_r\ (3)=10.13$, $p<0.002$. Five hours after withdrawal, none of the motor performance measures differ significantly from their respective baselines. At 10 hours, no measures differ from baseline except dyskinesia which is slightly lower ($p<0.05$). At 28 hours, dyskinesia, in-band efficiency, and work rate are all significantly disrupted at the 0.05 level.

DISCUSSION

The results indicate that withdrawal from drinking the low concentration of ethanol did not disrupt discriminative motor control performance. This is of interest because the actual

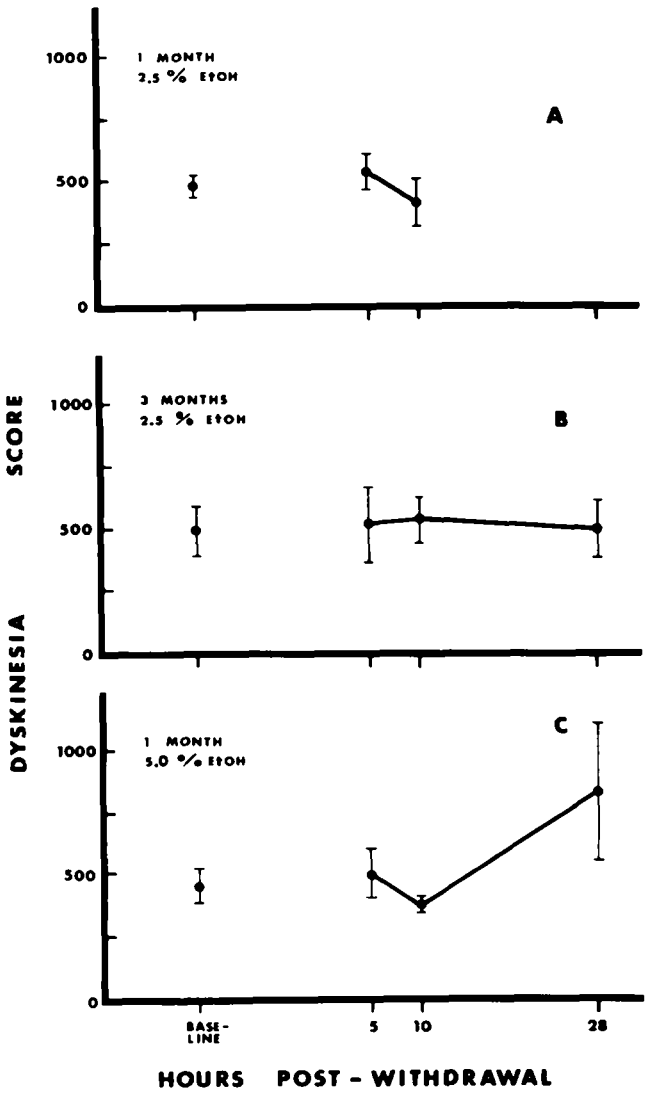

FIG. 1. Mean (± SE) dyskinesia score (number of entrances of applied force into the reinforced force band) for baseline (mean of the 4 sessions preceding EtOH withdrawal), and 5, 10 and 28 hr post-withdrawal. N=4 rats. *Dyskinesia score* increases as animal becomes tremulous and repeatedly exits from required force band.

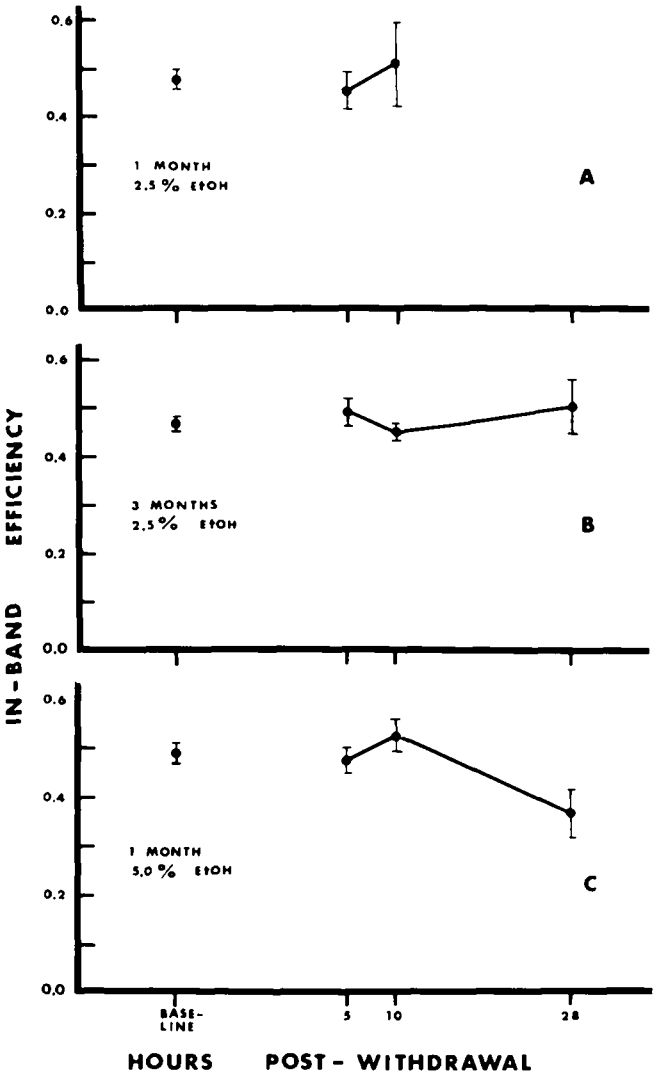

FIG. 2. Mean (± SE) in-band efficiency (minimum possible in-band time/actual time in-band) for baseline (mean of the 4 sessions preceding EtOH withdrawal), and 5, 10 and 28 hr post-withdrawal. N=4 rats. *In-band efficiency* declines from a perfect value of 1.0 as a function of an increasing number of in-band episodes that fall short of the reinforcement criterion (1.5 sec continuously in-band).

amount of ethanol drunk daily was only about 2.1 g/kg less than was the case when the higher concentration was made available. Yet, withdrawal from this greater concentration confirmed our previous finding of withdrawal dyskinesia [8]. The difference in blood ethanol level occasioned by this ethanol intake difference, however, was marked (see Table 1) and probably indicates that appreciable blood ethanol levels must be attained for withdrawal dyskinesia to develop.

The answer to the question of how high the blood ethanol level must rise and for how long for physical dependence to develop is probably a function of how dependence is defined and measured. Certainly, even a single, large dose can result in withdrawal signs [6]. French and Morris [5] found that rats maintained in a low ethanol vapor concentration environment in which blood ethanol levels remained undetectable, nevertheless showed an increased jump force response to electric shock after withdrawal compared to a dextrose control group. This result is consistent with the observation of an increased level of activity after withdrawal from an ethanol regimen in which rats drank amounts comparable to those consumed during our low ethanol intake phase [1].

It is possible that studies finding evidence of physical dependence after withdrawal from rather low levels of ethanol intake [1,5] differ from the present finding because our animals were performing an operant task. Those stuides reporting positive withdrawal signs after discontinuing low-level ethanol intake have employed unconditioned response measures. Perhaps our animals were more behaviorally tolerant to the effects of withdrawal owing to the strong stimulus control [2] exerted by the discriminative motor control schedule.

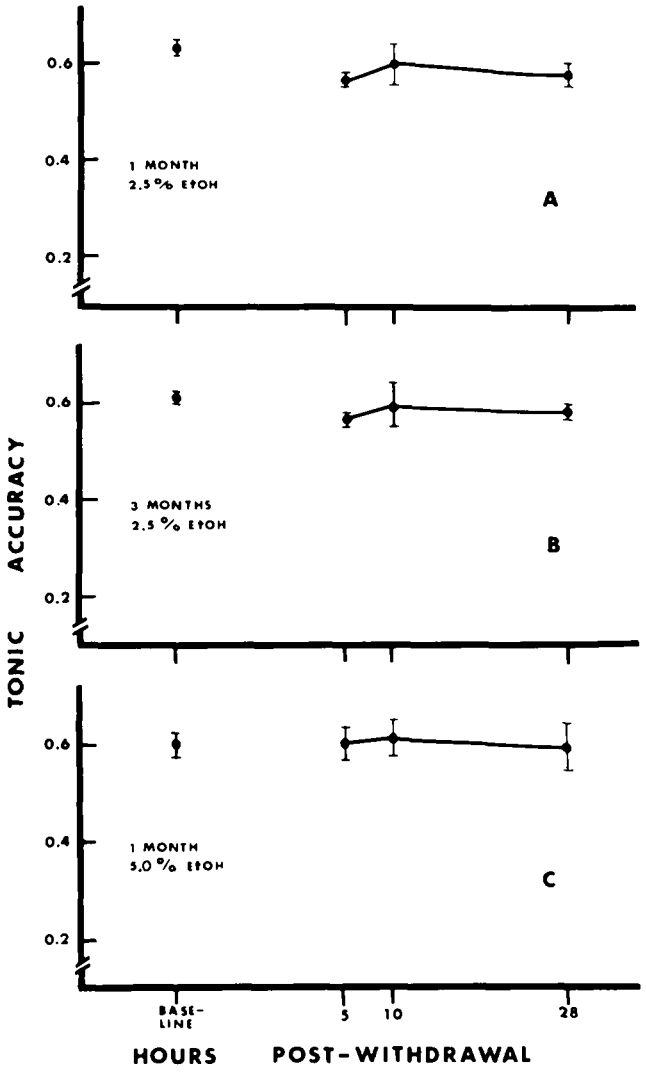

FIG. 3. Mean (± SE) tonic accuracy (in-band time/total response time) for baseline (mean of the 4 sessions preceding EtOH withdrawal), and 5, 10 and 28 hr post-withdrawal. N = 4 rats. *Tonic accuracy* gives the proportion of the total response time spent in-band.

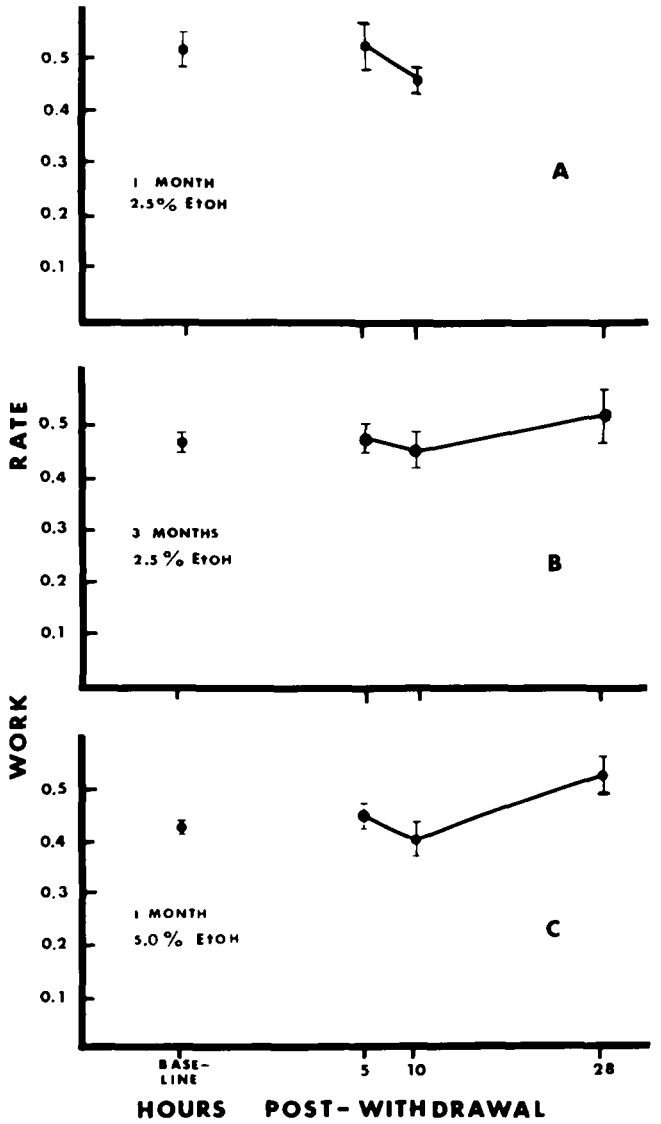

FIG. 4. Mean (± SE) work rate (total response time/session time) for baseline (mean of the 4 sessions preceding EtOH withdrawal). N = 4 rats. *Work rate* is the proportion of the session time spent responding on the force transducer.

REFERENCES

1. 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.
2. Dews, P.B. Behavioral tolerance. In: *Behavioral Tolerance: Research and Treatment Implications*, edited by N.A. Krasnegor. Washington, D.C.: NIDA Monogr. 18, 1978, pp. 18–26.
3. Falk, J. L. Drug effects on discriminative motor control. *Physiol. Behav.* **4:** 421–427, 1969.
4. Falk, J.L., H.H. Samson and G. Winger. Behavioral maintenance of high blood ethanol and physical dependence in the rat. *Science* **177:** 811–813, 1972.
5. French, S. W. And J. R. Morris. Ethanol dependence in the rat induced by non-intoxicating levels of ethanol. *Res. commun. chem. pathol. Pharmac.* **4:** 221–233, 1972.
6. Goldstein, D. B. Relationship of alcohol dose to the intensity of withdrawal signs in mice. *J. Pharmac. exp. Ther.* **180:**203–215, 1972.
7. LeBlanc, A. E. Microdetermination of alcohol in blood by gas-liquid chromatography. *Can. J. Physiol. Pharmac.* **46:**665–667, 1968.
8. Samson, H. H. and J. L. Falk. Ethanol and discriminative motor control: effects on normal and dependent animals. *Pharmac. Biochem. Behav.* **2:**791–801, 1974.
9. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill, 1956.
10. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.