between these two treatment groups.

Microfilaments are a second major class of cytoskeletal components present in these neurites. Their essential roles have been shown to be at the level of growth-cone activity, microspike formation, and collateral neurite development (*7*). The possibility that cytochalasin B might act specifically on these microfilaments (*7*) prompted us to attempt a similar reversal of its destructive action with dibutyryl cyclic AMP. In these experiments growth-cone activity was not restored in cultures treated with a maintenance dose of cytochalasin B (4 μg/ml, Imperial Chemical Industries), nor was neurite elongation stimulated significantly.

It is suggested that one action of dibutyryl cyclic AMP on the cytoskeletal elements of developing axons is at the level of the microtubules. Since the initial stages of nerve cell elongation have been reported to be independent of protein synthesis (*8*) it may be that dibutyryl cyclic AMP acts by stimulating microtubule assembly from a subunit pool, rather than by derepressing microtubular protein synthesis; this possibility is currently under investigation.

Our previous speculation that nerve growth factor (NGF), an agent known to stimulate axonal development, might in some way act through a second messenger system (*3*) led to similar experiments in which NGF was applied to Colcemid treated cultures. Nerve growth factor has in every case significantly ($P < .01$) reversed Colcemid's inhibitory effects (*9*). Therefore, in accordance with our hypothesis, one way NGF stimulates axonal maturation is apparently via a cyclic AMP intermediary which stimulates the assembly of microtubules from a *preexisting* subunit pool.

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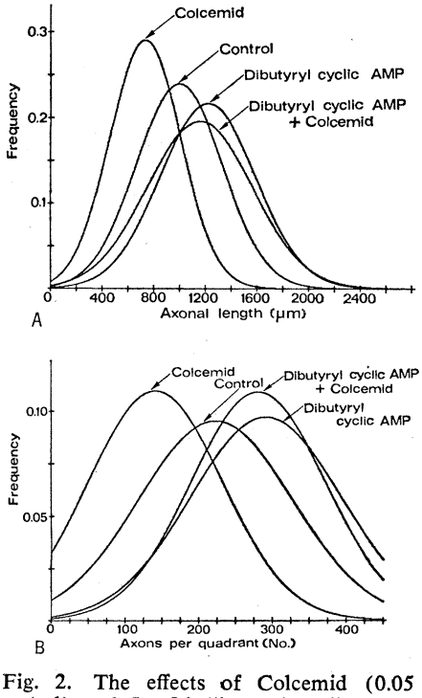

Fig. 2. The effects of Colcemid (0.05 μg/ml) and 5 m*M* dibutyryl cyclic AMP and of both together on axonal maturation are summarized in these normalized frequency distribution histograms based on a minimum of 60 cultures per group: (A) axonal length; (B) axonal number.

1 SEPTEMBER 1972

References and Notes

1. A. W. Hsie and T. T. Puck, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 358 (1971); G. S. Johnson, R. M. Friedman, I. Pastan, *ibid.*, p. 425.
2. P. Furmanski, D. Silverman, M. Lubin, *Nature* **233**, 415 (1971).
3. F. Roisen, R. Murphy, M. Pichichero, W. Braden, *Science* **175**, 73 (1972).
4. M. P. Daniels, *J. Cell Biol.* **39**, 31a (1968); F. J. Roisen and L. I. Rebhun, *Physiologist* **14**, 220 (1971).
5. K. Porter, in *Principles of Biomolecular Organization*, G. E. W. Wolstenholme and M. O'Connor, Eds. (Churchill, London, 1966).
6. F. Roisen, M. Pichichero, R. Murphy, W. Braden, *J. Neurobiol.*, in press.
7. K. M. Yamada, B. S. Spooner, N. K. Wessells, *J. Cell Biol.* **49**, 614 (1971); *Proc. Nat. Acad. Sci. U.S.A.* **66**, 1206 (1970).
8. N. W. Seeds, A. Gilman, T. Amano, M. Nirenberg, *Fed. Proc.* **29**, 2733 (1970); K. M. Yamada and N. K. Wessells, *Exp. Cell Res.* **66**, 346 (1971).
9. M. Pichichero, R. Murphy, F. Roisen, in preparation.
10. Supported by NIH general research support grant FR 05576.

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Behavioral Maintenance of High Concentrations of Blood Ethanol and Physical Dependence in the Rat

Abstract. *Rats maintained on an intermittent food schedule with an available ethanol solution drink to excess (13.1 grams of ethanol per kilogram of body weight, daily). Removal of ethanol produces symptoms of physical dependence including death from tonic-clonic seizures. Overindulgence in oral self-administration of an aqueous ethanol solution, resulting in unequivocal physical dependence, approximates a model of human alcoholism.*

The study of alcoholism would be facilitated if an animal model, possessing the major behavioral and physiological features of the human alcoholic, was developed. Recent reviews indicate (*1*) that presently available experimental arrangements fall short of providing such a model. One problem has been that the salient behavioral requirements of the model are quite demanding: (i) animals should orally ingest ethanol solutions excessively and chronically in a pattern that increases the concentration of blood ethanol analogous to that in the alcoholic; (ii) unequivocal physical dependence on ethanol must be demonstrated; (iii) food and ethanol should be available from sources physically separate so that the factors determining ethanol intake are not inextricably bound to those primarily concerned with meeting nutritional requirements; (iv) the experimental arrangement should retain an elective aspect to the ethanol ingestion by not programming extrinsic reinforcing events (for example, shock avoidance, food pellet delivery) contingent upon drinking ethanol. The technique we now describe satisfies all the above criteria.

Previously, one of us (*2*) found that volumes of water three to four times the normal 24-hour amounts were ingested when small food pellets were delivered intermittently to rats on a limited-food regimen. Because there is no increased fluid requirement in this method, polydipsia induced by a food schedule has been the subject of further research and theoretical speculation (see *3*).

Using the schedule-induced polydipsia technique, several investigators (*4*) have presented an ethanol solution as the available fluid instead of water. This research yielded no evidence of physical dependence on ethanol, perhaps because only single daily sessions, usually between 1 and 3 hours in length, were administered. Two studies, however, utilized longer sessions. Lester (*5*) maintained a rat on an excessive level of ethanol ingestion for a single 65-hour period. Ogata *et al.* (*6*) maintained mice on schedule-induced polydipsia for ethanol solutions during a 1- to 2-week period, but found no indications of physical dependence upon withdrawal of ethanol.

Eight male Holtzman rats, with a mean free-feeding body weight of 315.9 g, were reduced to 80 percent of their weights by limiting food rations (Purina Laboratory Chow, pelleted). They were housed in individual chambers under constant illumination. Each

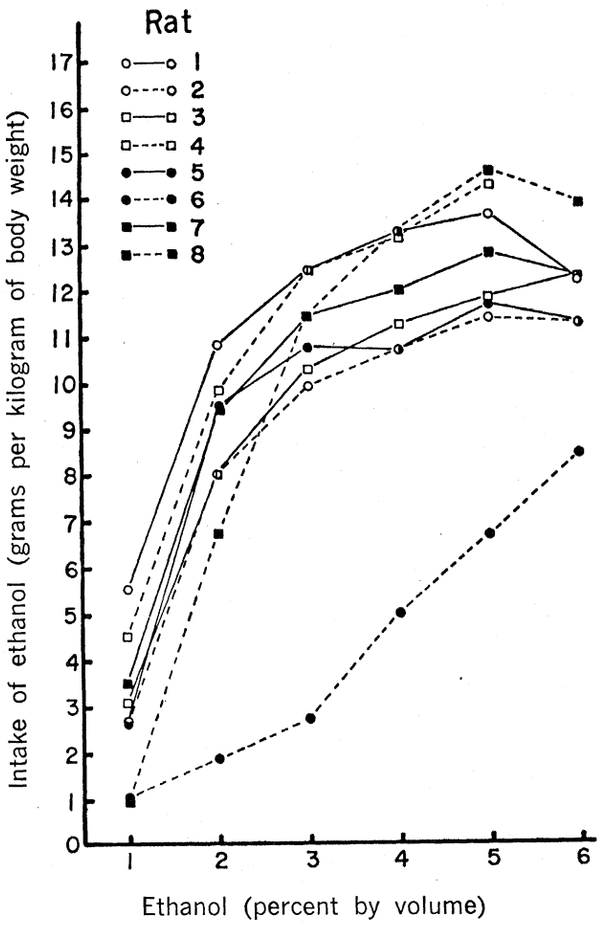

Fig. 1. Mean daily amounts of ethanol drunk by individual rats as a function of the available ethanol concentration.

chamber provided a source of fluid, accessible from a stainless steel drinking tube. A Noyes (*7*) animal food pellet (45 mg) was delivered automatically every 2 minutes during 1-hour feeding periods that were separated by 3-hour intervals. Thus, there were six feeding periods in each 24-hour cycle, delivering a total of 180 pellets.

In the first phase of the experiment, water was the available fluid. Each day, at 10:30 a.m., the fluid intakes were recorded, and the animals were weighed, and given any additional food necessary to maintain their weights at the 80 percent level. After the establishment of schedule-induced polydipsia, increasing concentrations of ethanol were substituted for water. Starting with ethanol 1 percent (by volume), the concentration was increased in 1 percent increments every 6 to 8 days until the drinking fluid was 6 percent ethanol. One animal (No. 4) was given a maximum concentration of only 5 percent ethanol.

The mean daily water intake at the end of the initial phase of the experiment was 64.0 ml (standard error of the mean, ± 12.3 ml). Figure 1 shows the results of the incremental-alcohol phase in terms of the mean daily self-administered dose of ethanol. One animal (No. 6) failed to develop polydipsia as strongly as the others whose daily intakes at 6 percent ethanol were between 11 and 15 g of ethanol per kilogram of body weight. Because the intake of ethanol (in grams) did not increase when the concentration was changed from 5 to 6 percent, the fluid concentration was returned to 5 percent for the next 3 months, after which time the experiment was terminated. When the animals began drinking alcohol, additional food supplements were omitted for the remainder of the experiment as the animal weights were increasing.

During the early phases of ethanol drinking, the animals appeared to be intoxicated when handled at 10:30 a.m.; they were docile and somewhat ataxic. Later, presumably as tolerance developed, gross observation failed to reveal these or other signs of unusual behavior.

During their second month on 5 percent ethanol, the concentrations of ethanol in the blood of all rats was measured (*8*). Blood samples (50 μl) from the tail were taken 1 hour before and 1 hour after each of the 1-hour feeding periods. Serial samples were taken first between 8 a.m. and 7 p.m.; 2 weeks later, the samples between 8 p.m. and 7 a.m. were gathered. Figure 2 shows that for most animals, the concentration of ethanol in the blood fell below 100 mg/100 ml only in the late morning and afternoon. From 6 p.m. through 11 a.m., the concentration was greater than 100 mg/100 ml for most of the animals, and was often between 150 and 300 mg/100 ml.

Daily intake of ethanol remained quite constant during the 3-month period on 5 percent ethanol. For the last 10 days of the experiment, the mean daily intake for the eight animals was

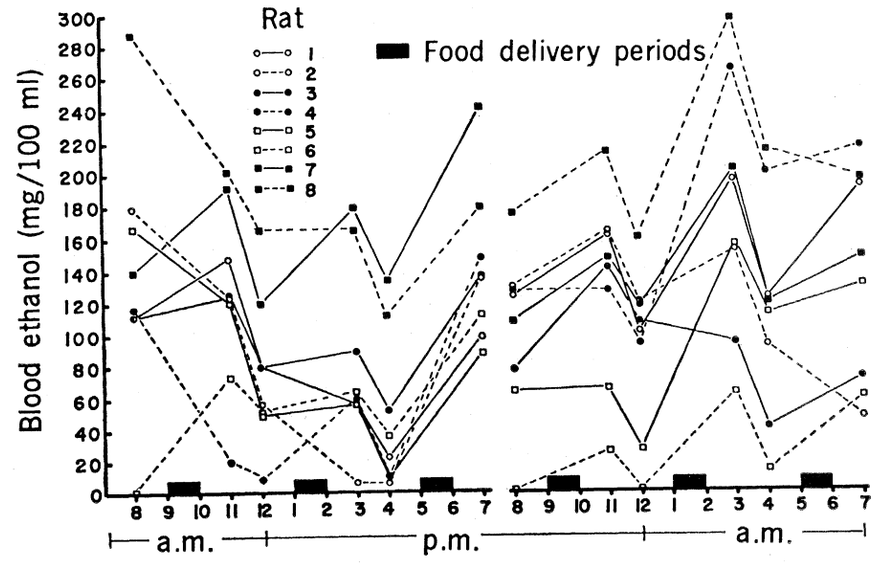

Fig. 2. Concentrations of ethanol in the blood of rats drinking 5 percent ethanol (by volume). During the indicated 1-hour feeding periods, a food pellet (45 mg) was delivered every 2 minutes.

13.1 g of ethanol per kilogram of body weight, with a standard deviation of 1.29. The percentage of the total caloric intake which was ingested as ethanol was 44.8 percent. The mean body weight of the group was 308.2 g, which was not significantly different from the free-feeding starting weight of 315.9 g.

Three to four weeks from the end of the experiment, we selected four animals for observation during alcohol withdrawal. They were removed from the experimental cages at 7 a.m., and were placed in individual observation cages with water, but no alcohol, available. Food rations, in amounts equivalent to those defined by the experiment, were given at the appropriate times. Within 3 to 4 hours after the last feeding period (5 to 6 a.m.), when most of their last draughts of ethanol presumably occurred, the animals became hyperactive. A shaking of keys near the top of the cage for 1 to 2 seconds resulted in a tonic-clonic convulsion in rat No. 8. For the next hour, tremors, spasticity, and clonic head movements occurred, and finally, a second seizure ended in death. When keys were shaken (2 to 5 seconds) for the first time after 9½ hours of withdrawal, a clonic running episode was produced in rat No. 2, followed shortly by death from a tonic-clonic seizure. Rat No. 7 showed all the preconvulsive symptoms, but keys shaken (up to 20 seconds) after 15 hours of withdrawal had no effect. Rat No. 1 was similar, but no attempt was made to trigger a convulsion by shaking keys. Attempts to produce convulsions in normal Holtzman rats by prolonged key shaking were unsuccessful, and no preconvulsive, hyperactive behavior was observed.

We are unaware of any previous report demonstrating physical dependence on ethanol in the rat as indicated by withdrawal convulsions, although this has been obtained in other species, as well as in man (*9*). We could also find no previous report of the development of physical dependence by self-administration in animals other than man when ethanol in water was available as the sole drinking solution.

Other methods for the production of ethanol dependence in animals have involved administration by intravenous and intragastric routes, by liquid diets, and by inhalation (*9, 10*). While useful, these methods have certain disadvantages; they are more removed from a model of alcoholism involving oral self-administration of an aqueous ethanol solution than is the method reported here. In animals consuming liquid diets in which 35 to 36 percent of the calories are derived from alcohol, the concentrations of ethanol in the blood appear to be rather low (*10*) unless the animals suffer considerable concomitant weight loss with presumed changes in their capacity to metabolize ethanol (*6*). The present method maintains high concentrations in the blood without significant body weight loss.

The percentage of total caloric intake derived from ethanol is low in most experiments utilizing ethanol in the drinking water. One study reports a caloric intake from ethanol as high as 30 percent (*11*). Attempts to incorporate 45 percent of the calories as ethanol into a liquid diet resulted in death of the animals (*12*). The 44.8 percent caloric intake in the present experiment compares favorably with this picture, and with the amount selected by human alcoholics (*13*).

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References and Notes

1. N. K. Mello, in *Psychopharmacology. A Review of Progress 1957–1967*, D. H. Efron, Ed., Publ. 1836 (Government Printing Office, Washington, D.C., 1968), p. 787; R. D. Myers and W. L. Veal, in *The Biology of Alcoholism*, H. Begleiter and B. Kissin, Eds. (Plenum, New York, 1971), vol. 2, p. 131.
2. J. L. Falk, *Science* **133**, 195 (1961).
3. ———, *Physiol. Behav.* **6**, 577 (1971).
4. R. B. Holman and R. D. Myers, *ibid.* **3**, 369 (1968); R. A. Meisch and T. Thompson, *Psychopharmacologia* **22**, 72 (1971); N. K. Mello and J. H. Mendelson, *Physiol. Behav.* **7**, 827 (1971); R. J. Senter and J. D. Sinclair, *Psychon. Sci.* **9**, 291 (1967).
5. D. Lester, *Quart. J. Stud. Alc.* **22**, 223 (1961).
6. H. Ogata, F. Ogata, J. H. Mendelson, N. K. Mello, *J. Pharmacol. Exp. Ther.* **180**, 216 (1972).
7. Composition according to P. J. Noyes Co. is: moisture, 5.0 percent; ash, 6.3 percent; ether extract, 4.9 percent; protein, 25.2 percent; fiber, 3.3 percent; carbohydrates, 55.3 percent; 4.3 kilocalories per gram.
8. N. G. Brink, R. Bonnichsen, H. Theorell, *Acta Pharmacol. Toxicol.* **10**, 223 (1954).
9. G. Freund, *Arch. Neurol.* **21**, 315 (1969); C. F. Essig and R. C. Lam, *ibid.* **18**, 626 (1968); F. W. Ellis and J. R. Pick, *J. Pharmacol. Exp. Ther.* **175**, 88 (1970); G. Deneau, T. Yanagita, M. H. Seevers, *Psychopharmacologia* **16**, 30 (1969); D. B. Goldstein and N. Pal, *Science* **172**, 288 (1971).
10. C. S. Lieber, D. P. Jones, L. M. DeCarli, *J. Clin. Invest.* **44**, 1009 (1965).
11. R. Scheig, N. M. Alexander, G. Klatskin, *J. Lipid Res.* **7**, 188 (1966).
12. G. Freund, *J. Nutr.* **100**, 30 (1970).
13. J. H. Mendelson and J. LaDou, *Quart. J. Stud. Alc.* Suppl. 2, 14 (1964).
14. Supported by NIMH grant MH 18409 from the National Institute of Alcohol Abuse and Alcoholism, and NIH grant AM 14180.

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Chromatic Specificity of the Visual Evoked Response

Abstract. *The temporal alternation of red and green stripes in a structured field produces successive contrast, which can elicit cortical potentials recorded from the scalp. Amplitudes of the major frequency components of the potentials correspond to the relative intensities of red and green producing the contrast. The amplitude variations are color-specific, since total luminance and structure are held constant.*

In studies of cortical processing of color in man, the stimulus procedure used to elicit the visual evoked response (VER) has been the spatial and temporal alternation of two colors presented in a striped or checkerboard field (*1, 2*). The major advantage of the alternation procedure is that chromaticity can be varied independently of luminance, which remains constant. An assumption has been that contribution of the two colors to the response is equal or constant. For example, red and green alternated 180° out of phase (that is, the colors exchanging places at a given frequency) produce a presumably comparable response to each alternation regardless of direction of color change. Thus, the response is a difference measure between the two colors, and a response to a given color per se is not available.

Our purpose here is to demonstrate that with temporal alternation alone (successive contrast) in a structured field, responses to red-to-green alternations are different from those to green-to-red alternations. This previously unreported effect (*2, 3*) represents a fundamental difference between the red and the green processing channels. This difference is apparent because their respective contributions to the response are not equal, and thus a degree of chromatic specificity is obtained. We also show that VER differences between the red and green channels are independent of luminance.

Color stimuli were produced by narrow-band interference filters (Baird-Atomic) in a Maxwellian view system. For monocular stimulation, two channels presented a field of 20° to the subject's right eye, with central fixation aided by a bite bar. Two identical channels for the left eye were available for binocular stimulation. Within the field, subjects with normal color vision saw