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16. We thank Dr. H. T. Openshaw, Wellcome Research Laboratories, Beckenham, Kent, England, for supply of tetrahydropapaveroline (1,2,3,4-tetahydro-6,7-dihydroxy-l-(3′,4′-dihydroxybenzyl)-isoquinoline hydrobromide). Supported by PHS grant MH 15814.

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Cretinism in Rats: Enduring Behavioral Deficit Induced by Tricyanoaminopropene

Abstract. *Rats reared on diets containing tricyanoaminopropene, the antithyroid compound that stimulates RNA synthesis, showed a deficit in performance on automated closed-field maze tests many weeks after discontinuation of the drug. The rats were also tested while still receiving the drug, and performance deficits were indicated in tests of Y-maze reversal and manual closed-field maze performance; rats treated with the drug and with thiouracil behaved in a highly similar fashion on several tasks. No evidence of facilitation by tricyanoaminopropene appeared in any of the eight learning situations used. Exposure to tricyanoaminopropene before and after birth, at doses sufficient to produce anatomical cretinism, apparently induces an enduring behavioral deficit which is similar to that of neonatal thyroidectomy-induced cretinism in rats and which parallels the mental retardation associated with human cretinism.*

The compound 1,1,3-tricyano-2-amino-1-propene (TCAP) is of interest to those studying biochemical processes in learning and memory primarily because of its reported stimulation of brain RNA and protein synthesis (*1, 2*). Several investigators (*2–4*) have reported facilitative effects of TCAP in learning situations, but conflicting reports (*5, 6*) have also appeared, and thus the status of TCAP as a "learning facilitator" is in doubt.

The antithyroid properties of TCAP are more firmly established than its RNA- and protein-stimulating effects. Ingbar (*7*) has shown that in rats TCAP inhibits the organic binding of iodine, the formation of thyroxine, the conversion of monoiodotyrosine to diiodotyrosine, and the action of the thyroidal iodide-concentrating mechanism; others (*8*) have reported that, in rats treated with TCAP, concentrations of protein-bound iodine are reduced and ultrastructural changes in thyroid tissue occur which are similar to those produced by other antithyroid compounds. When the drug is fed over a long period to immature rats, beginning before or after birth, the typical anatomical signs of cretinism, including dwarfism, altered skeletal development, and persistence of immature hair and skin features, appear.

Better than normal performance has been reported (*4*) in rats with cretinism induced by TCAP in both the original learning and reversal of an automated Y-maze discrimination, in contrast to apparent learning deficits (*9*) for rats treated with thiouracil and those with thyroidectomy-induced cretinism. The Y-maze testing was done when the rats treated with TCAP were receiving the drug and were partially deprived of food, conditions which could have generated motivational confounding. Up until now no tests of the effects of early long-term TCAP administration have been conducted in a learning situation after discontinuation of the drug.

In two experiments, rats were made cretinoid by diets of TCAP given before and after birth and were tested on a variety of learning tasks, some of which (on-drug tests) were given during early months when physical growth was arrested by maintenance of the drug, and others (post-drug tests) were given after the drug was discontinued. In experiment 1, the behavior of rats reared on a TCAP dose (1.5 g per kilogram of mash) (*4*) was compared with that of control rats reared on plain mash. In experiment 2, the effects of a lower (1.0 g per kilogram of mash) dose of TCAP, a dose of thiouracil (1.0 g per kilogram of mash), and a control diet were compared.

In both experiments, pregnant albino rats (Holtzman Co.) were started on the experimental diets on day 5 of gestation. The rats were given free access to experimental and control regimens (*10*) throughout the gestation and lactation periods and in the offspring's maintenance after weaning until the ages of 130 (experiment 1) or 212 (experiment 2) days (*11*). Physical growth of the offspring fed TCAP and thiouracil, in terms of body weight, leveled off within the first 80 days. Weights in the female offspring treated with the drugs at 60 days of age averaged 73 g for the group fed 0.15 percent TCAP, 78 g for the group fed 0.1 percent TCAP, and 56 g for the group fed 0.1 percent thiouracil; weights of female controls at that age were 185 g. More pronounced dwarfism occurred in the male groups given TCAP.

When the drugs were discontinued, all animals were then given free access to a Purina chow diet. Regrowth in the cretinoid rats took place on the latter diet and, in terms of nearly asymptotic body weights, amounted to 83 percent (experiment 1) and 73 percent (experiment 2) of control weights in the females fed TCAP and 71 percent of normal in the females fed thiouracil. During the tests after discontinuation of the drug, the animals were maintained at 85 percent of terminal regrowth weights.

In experiment 1, the on-drug tests consisted of discrimination and seven reversals in a Y-maze followed by tests in a Rabinovitch-Rosvold closed-field maze (*12*). The post-drug tests were (in order): bar-pressing acquisition, extinction, and reacquisition; multiple-choice performance in four-lever operant chambers; four additional Y-maze reversals; running wheel activity; runway discrimination; and automated symmetrical maze tests (*13*). The rats in experiment 2 received on-drug tests of running wheel activity, Y-maze discrimination and reversal, and runway discrimination and post-drug tests of symmetrical maze performance and

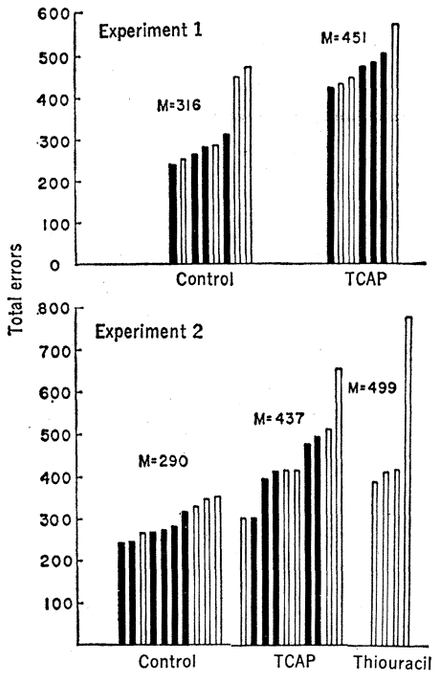

Fig. 1. Mean (M) numbers of errors for the 12 problems for individual rats in the automated symmetrical maze tests. Solid bar, male rats; clear bar, female rats.

conditional (sign-differentiated spatial) discrimination learning.

In the on-drug tests neither experiment confirmed the finding (*4*) that TCAP facilitated Y-maze performance. These tests also disclosed strong evidence of motivational confounding which has led us to discount findings of learning facilitation obtained in on-drug tests of early long-term TCAP administration in general and the Schmidt and Davenport result in particular. Such findings may merely represent enhancement of performance in animals treated with TCAP by usually high motivation stemming from the stimulant properties of the drug and from desuppression of thyroid function when the drug is withheld during food deprivation intervals (*14*).

In experiment 1 the rats fed TCAP showed significantly poorer performance than control rats in the first reversal of the Y-maze task, directly contradicting Schmidt and Davenport (*4*), and in the Rabinovitch-Rosvold closed-field maze tests (*15*). In these two instances the poorer performance by these rats occurred in spite of the motivational advantages they may have held over the control rats and thus may be interpreted as indicative of learning deficits.

The post-drug tests were presumably free from confounding motivational factors, and this presumption was supported by an absence of group differences in bar-pressing rates, activity scores, and response speeds. Of the various post-drug tests administered, only the automated, symmetrical maze tests disclosed significant differences in learning rate. However, striking differences between the former cretinoid rats and the controls were revealed by the symmetrical maze tests in both experiments, long after drug administration had been discontinued (31 weeks in experiment 1, 15 weeks in experiment 2). Many more errors were made by the rats fed TCAP and thiouracil than by the controls, and statistical analyses (*16*) showed comparisons of drug to control data to be highly significant (Fig. 1). No important difference between the females fed TCAP and thiouracil in experiment 2 occurred. When the data of all the early hypothyroid rats of both experiments were combined and compared with the control data, the mean error scores were numerically higher for the hypothyroid rats on each of the 12 symmetrical maze problems [fig. 4 in (*31*)].

In summary, no learning task in either experiment disclosed any evidence of facilitation by TCAP, and three tasks provided evidence of deficit attributable to TCAP. In view of the agreement between the two experiments in the symmetrical maze findings and the similar effects of TCAP and thiouracil in all the tasks of experiment 2, it seems that the early long-term administration of TCAP induced enduring and probably permanent deficits in certain types of learning capacity because of central nervous system arrest resulting from the drug's antithyroid action at prenatal or neonatal stages of development. Although such arrest has not yet been demonstrated neurohistologically in the case of TCAP, this interpretation is in harmony with the major facts of human cretinism and with the conclusion (*9*) that neonatal thyroid deficiency induces irreversible behavioral deficit and permanent arrest of neural maturation in the rat.

These findings, however, do not rule out the possibility that TCAP may facilitate learning and memory when administered in such a manner as to avoid early thyroxine deficiency. Reports (*2, 3*) of memory facilitation by TCAP in older animals are thus not contradicted.

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10. Pair-fed controls were not run because there was no evidence of reduction of food intake or of body weights of the pregnant rats when the rats were fed mash treated with the drug during gestation.
11. The number of offspring at birth were 36 (TCAP) and 48 (control) in experiment 1 and 28 (TCAP), 28 (thiouracil), and 42 (control) in experiment 2, but attrition from cannibalism and insufficient physical development of the offspring reduced the number in the TCAP group to 16 (experiment 1) and 20 (experiment 2). All 28 offspring from the thiouracil group survived the lactation period, but only four females in this group survived the on-drug phase of experiment 2 after weaning.
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13. J. W. Davenport, W. W. Hagquist, G. R. Rankin, *Behav. Res. Meth. Instrum.*, in press. The symmetrical maze is similar to the Rabinovitch and Rosvold (*12*) apparatus, except for the use of motorized endbox doors, a bidirectional shuttling procedure, and a newly designed set of 12 maze patterns. Each pattern is a symmetrical arrangement of barriers within the field so that within a given problem the rat must make the same order of left and right turns to get from one endbox to the other regardless of which endbox is serving as the goalbox on a given trial.
14. That TCAP acts as a stimulant is indicated by Gurowitz (*6*) and L. Solyom and H. M. Gallay [*J. Neuropsychiat.* **2**, 577 (1966)]. This source of confounding in the on-drug tests was probably of lesser consequence than the procedure of depriving the rats of food (and thus also the drug) for 16 to 18 hours on each testing day, which was employed in the interests of replicating the Schmidt and Davenport (*4*) study. It seems probable that extreme metabolic needs were generated in the cretinoid rats by this deprivation procedure because the drug (TCAP or thiouracil) was no longer ingested in sufficient quantity and frequency to keep thyroid secretion suppressed. Activity scores and locomotion speed measures obtained in the on-drug phase were higher for the cretinoid rats than the controls in both experiments.
15. Mean numbers of errors to criterion in the first Y-maze reversal of experiment 1 for 12 rats fed TCAP (rewarded for correct responses by one 45-mg food pellet), 15 control rats rewarded by one pellet, and 15 control rats rewarded by three pellets were 57.6, 31.7, and 39.7, respectively. In *t*-tests following analysis of variance, the TCAP error mean was significantly above the one-pellet ($P < .001$) and three-pellet ($P < .05$) control means. In the Rabinovitch and Rosvold tests, a drug-by-sex interaction was found ($F = 5.91$; d.f. $= 2,20$; $P < .01$), reflecting the fact that significantly more errors per problem were made by the male rats fed TCAP (mean of 16.0) than by male controls rewarded with one pellet (11.6) and male controls rewarded with three pellets (11.0) while no significant difference among the female groups occurred.

16. In analyses of variance, there were significant main effects of drug groups in terms of total errors over the 12 problems (experiment 1, $F = 19.02$; d.f. $= 1,11$; $P < .005$; experiment 2, $F = 8.11$; d.f. $= 2,21$; $P < .01$) and mean trials to criterion (experiment 1, $F = 13.71$; d.f. $= 1,11$; $P < .005$; experiment 2, $F = 8.78$; d.f. $= 2,21$; $P < .01$). No significant interactions of drug with problems or with sex occurred. Subsequent tests in experiment 2 showed that both the groups fed TCAP and thiouracil made significantly ($P < .01$) more errors than the controls.

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Directional Hearing: Effect of Unilateral Change of the Sound Duration

Abstract. *Monaural change of the duration of a binaural acoustic stimulus within the range of 0.5 to 1.5 milliseconds caused a considerable shift of the sound image. This could be counterbalanced by a unilateral change of the signal intensity. Further unilateral lengthening of the stimulus did not affect the sound localization, but it revealed a masking aftereffect (4 to 10 milliseconds) of the binaural signal. The data define the temporal characteristics of binaural effect formation and the relative importance of duration and intensity parameters at different stages of binaural interaction.*

Intensity difference of the acoustic signal in the right and the left ear is one of the main factors determining sound localization. In this study I have attempted to substitute the relative sound duration for this factor.

Bursts of white noise of equal loudness (5 or 25 db above the auditory threshold) and equal initial duration (from 0.7 to 70 msec) were presented through earphones simultaneously to both ears of human subjects; in this way a single sound image was created, perceived on the midline of the head. After several presentations, the duration of the signal at one ear was increased or reduced by steps (Fig. 1), while at the other ear it remained unchanged. The subjects were instructed to report after each stimulation whether they perceived the sound strictly on the midline, or to the right, or to the left.

In 160 experiments on 12 subjects unilateral changes in stimulus duration within the range of 0.1 to 1.5 msec produced a significant (P from .001 to .02) shift of the sound image in the direction of the longer stimulus. This duration effect could be counterbalanced, that is, the sound could be brought back to the midline, by changing the intensity of the simultaneous stimulation of the other ear. The compensatory change in intensity (in decibels) served as a measure of the lateralization of the sound image (Fig. 1).

The extent of the lateralization did not depend on the initial loudness (5 or 25 db). The relative duration proves to be a very effective factor of the binaural interaction. It is much more effective than is relative intensity. Thus, a unilateral reduction of duration from 1.0 to 0.6 msec, or by less than half, was counterbalanced by a change in the intensity of the contralateral stimulus by 20 db, or ten times, that is, by a much greater amount of total energy.

However, the efficiency of the duration factor, that is, of the temporal summation, is confined to a limit of about 1.5 msec. A further unilateral lengthening of the stimulus does not affect the position of the sound image —the lines become parallel to the abscissa (Fig. 1).

For other auditory functions the temporal summation is much longer. Thus, the lowering of the auditory threshold and the augmentation of the loudness, caused by prolongation of the acoustic stimulus, do not cease until the duration of the stimulus exceeds 100 to 150 msec (*1*). But the binaural interaction is achieved, as it is seen, within 1.5 msec; the binaural effect is formed by this time, and later events cannot influence its formation.

It seems likely that the observed lateralization of sound is a manifestation of unilateral changes in auditory thresholds and, hence, in loudness which are brought about by monaural changes in sound duration. Indeed, the obtained lateralization values are very

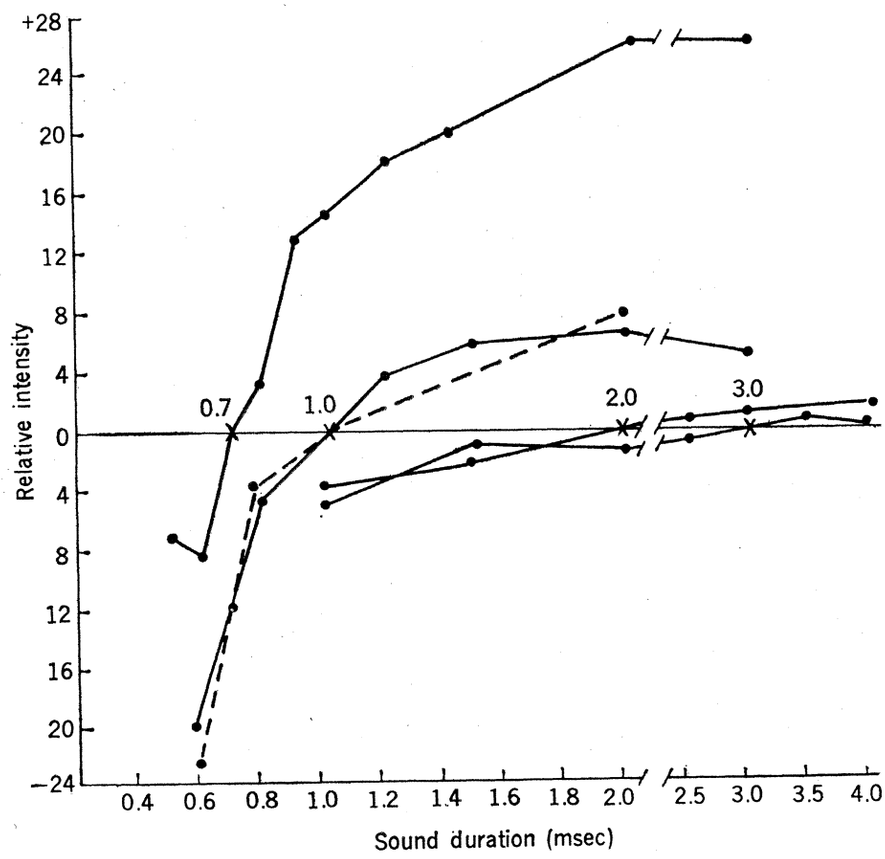

Fig. 1. Sound image shifts caused by unilateral changes of stimulus duration and compensated by an increase (+) or decrease (−) of the contralateral stimulus intensity. Crosses and figures on the base line represent initial sound durations. The dotted line represents auditory thresholds for sounds of different durations; the threshold for 1.0-msec stimulus is taken as a reference. Mean values.