we also studied *N*-dimethylaminosuccinamic acid, where the preferred orientation should be *trans;* this *trans* compound was stable in aqueous solution at 25.0°C for several days.

The capacity of CO11 to retard plant growth is probably due to the presence of 1,1-dimethylhydrazinium hydrogen maleate in the aqueous mixture and not solely to CO11 as suggested by Riddell and coworkers (*1*).

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Infant Handling: Effects on Avoidance Learning, Brain Weight, and Cholinesterase Activity

Abstract. *Infant rats were "handled" by removing them periodically from their home cages. "Non-handled" rats were left undisturbed. Half of the animals were killed at weaning, and weights and cholinesterase activity were determined on four different sections of the brain. The remaining animals were conditioned to avoid an administered shock. Handling increased ventral-cortex and subcortical weights and decreased subcortical cholinesterase. No differences in avoidance conditioning were observed.*

Many experiments demonstrate that a rat's experience early in life affects its later behavior. Several procedures have been used to manipulate early experience, all of which have in common the removal of the animal from its home cage. These preweaning experiences result in animals that are less emotional in open field tests. Thus, they show less defecation and urination and a greater degree of exploration in a strange environment (*1*), and they are better able to learn a conditioned avoidance response (*2*). Raising animals in a complex environment after weaning results in animals that are more capable of learning complex tasks, such as making fewer errors in a maze (*3*) and in a discrimination-learning situation (*4*).

In few of these investigations have the related changes in the nervous system which result from these experiences been studied. In one promising line of research animals raised in conditions which provided a large amount of environmental stimulation during growth showed differences in brain weight and brain cholinesterase activity from littermates raised in restricted environments (*5*). These experiments have manipulated the experience of the animals after weaning. The purpose of our experiment was to determine the effects of handling before weaning on avoidance conditioning, the distribution of brain weight, and brain cholinesterase activity.

The subjects for this experiment were 58 descendants of the Tryon S_3 strain that were littered by six female rats. Before the pups were born each mother was assigned at random to either the control or the experimental group. The control litters were not disturbed until weaning. The experimental litters were handled once daily from age 2 to 10 days. The handling procedure consisted of removing the infant rats from the home cage and placing them in a metal pan partly filled with wood shavings for 5 minutes. At the end of this period the pups were returned to the home cage. After the ninth day of handling the experimental animals were not disturbed until weaning. None of the cages were changed throughout the course of the experimental treatment, and care was taken not to stimulate the animals unnecessarily.

At 25 days of age the animals were removed from the home cage, their ears were punched for identification purposes, and approximately half the animals of each sex from each litter were killed for chemical analysis. The remaining animals were kept for avoidance training and were housed by sex and by litter.

The animals designated for chemical analysis were decapitated, the brains were dissected, and samples of tissue were removed by a standardized gross dissection procedure (*6*). The tissues analyzed were from the visual and somesthetic areas of both cortical hemispheres, the remaining dorsal cortex, the ventral cortex, and the remaining brain (subcortex). Weights and cholinesterase activity of the visual and somesthetic areas were averaged and represent the sensory cortex. Immediately after dissection, the weight of each sample was determined to the nearest 0.01 mg with a direct-reading analytical balance. Samples were frozen on dry ice and stored at −20°C until they were analyzed.

Table 1. Means of cholinesterase activity and brain weights for handled (H) and nonhandled (NH) groups. Cholinesterase activity: 10^{10} moles of acetylcholine hydrolized per minute per milligram of tissue.

Brain weight (mg)		Cholinesterase activity	
H	NH	H	NH
Sensory cortex			
52	50	57	57
Remaining dorsal cortex			
259	248	56	61
Ventral cortex			
276	254*	84	90
Subcortical brain			
790	710*	170	182*

* $p < .05$.

Cholinesterase activity was assayed by the method of Rosenzweig, Krech, and Bennett (*7*). For avoidance training, a box was divided into two compartments, each 12 by 4 by 8 inches, separated by a guillotine-type door. The start compartment had a grid floor through which a 0.5-ma shock could be delivered to the animal's feet; the safe compartment had a wooden floor. The experimenter could observe the animal through Plexiglas lids on the top of each compartment.

A trial began when the center door was raised. This action closed a microswitch which simultaneously started a warning buzzer, a standard electric timer, and a time-delay relay which controlled the onset of the shock. Shocks were terminated when the animal entered the safe compartment and interrupted a photocell beam. Latency (the time between the opening of the door and the interruption of the photocell) was recorded after each trial. The interval between the onset of the buzzer and the onset of the shock was 5 seconds. A 30-second rest period was allowed in the safe compartment before the animal was returned to the shock compartment. A total of 40 seconds elapsed between the end of one trial and the beginning of the next.

The first day of avoidance training was begun when the animal had reached 30 days of age. The procedure for each of the four trials on this day was the same as that described, but no shock was delivered to the animal. A maximum latency of 60 seconds was allowed. If the subject had not crossed to the safe compartment in this time,

the trial was terminated and the next trial began 40 seconds later. Beginning the next day, all animals were subjected to five trials per day until there were two consecutive days in which no shock was received, or 10 successful avoidance trials in two days (criterion).

The data were analyzed by the procedures (*8*) for analysis of variance for a 2 by 2 factorial design with unequal numbers in each cell. Scores of the trials through criterion and the sum of the median latencies for the first four days were used to assess the effects of infant handling on acquisition of avoidance conditioning. The experimental group was composed of four males and six females. There were five males and 12 females in the control group. There were no significant differences between the handled and nonhandled groups or between the males and females, nor were there significant interactions on either measure.

These results, not in agreement with others (*2*), are probably attributable to differences in apparatus. Where infant handling facilitated avoidance learning the animals were required to open an escape door in order to leave the shock compartment. In our experiment, the animal learned only the simple response of running from one compartment to the other at the onset of the buzzer. Criterion was reached by 50 percent of the animals in 30 trials; the average for all animals was six errors during the first 20 trials. The escape door in the previous experiments may have inhibited responding by the more emotional nonhandled animals and thus differentially have affected the rate of acquisition. This explanation is substantiated by Levine's (*2*) observation that the nonhandled animals take a greater number of trials before making their first avoidance response.

The means of the brain weights and cholinesterase activity for the handled and nonhandled animals are presented in Table 1. These data are based on the six males and four female animals from the handled group and seven males and 14 females from the nonhandled group. The statistical analysis of the data indicated that sex was a significant main effect for the subcortex and dorsal-cortex weights and dorsal-cortex cholinesterase activity; the males showed higher weights and lower cholinesterase activity. The handling procedure produced significant increases in the weights of the subcortex and the ventral cortex and significant decreases in cholinesterase activity in the subcortex.

The results of the analysis of the brain weights and cholinesterase activity are more easily interpreted when considered with the experiments of Krech *et al.* (*5*) in which postweaning experience was manipulated. There, animals raised in enriched environments show greater weight and lower cholinesterase activity in the cortex than littermates raised in isolation. Subcortical cholinesterase tends to be higher for the enriched animals and there are no differences in subcortical weights.

The results of our experiment are in direct contrast to those of Krech *et al.* (*5*). The measures on the cortex do not differ between the handled and nonhandled groups but the subcortical measures do. These results imply that pre- and postweaning experiences produce different effects on brain weight and brain cholinesterase; the infant handling procedures affect the subcortical brain while postweaning environmental enrichment affects the neocortex.

The specific mechanisms which cause these alterations in brain weight and cholinesterase are unknown. These results do suggest that the behavioral changes associated with the infant handling and environmental enrichment procedures may be mediated by changes in the central nervous system that affect central cholinergic mechanisms (*9*).

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Mitoses: Distribution in Mouse Ear Epidermis

Abstract. *Epidermal cell mitoses occur in a random spatial (Poisson) distribution in the plane of the epidermis of the mouse. It is therefore unlikely that the focal accumulation of a local extracellular factor is responsible for the initiation of mitoses.*

When mitoses are counted in a tissue such as the epidermis, they sometimes seem to occur in clusters (*1*). This may be of some importance, for if mitoses indeed occur in clusters, it would suggest that some focal factor which acted on a group of cells was concerned in the initiation of cell division.

In order to test this hypothesis one ear of each of five anesthetized adult, Swiss male mice was removed with sharp scissors, and a 3 by 4 mm rectangle was then cut from the excised ear. These biopsies were placed immediately in 0.5 percent acetic acid at 4°C and incubated for 5 hours to allow separation of the epidermis from the dermis. The epidermis was then dissected off with a spear-point needle, stretched with straight pins on a piece of cork and fixed in 10 percent formalin overnight (*2*). The tissue was stained with haemalum (*3*), dehydrated in three changes of absolute alcohol, cleared in three changes of toluol, and mounted with the basal cell layer up. Such specimens, when examined with an oil-immersion lens, show epidermal cells and their mitoses clearly.

The preparations consist almost entirely of a single layer of basal cells covered by a very attenuated layer of flattened squamous cells. This two-dimensional tissue is well suited to a study of the distribution of mitoses. The proportion of cells in mitosis was determined in each specimen by means of an ocular grid in a square area of 100 grid units, each unit containing an average of 700 to 800 nuclei. Because of Gelfant's (*4*) observation that there is an increased mitotic frequency near the cut edge in such biopsies, only the areas distant from the cut edges were counted, even though the immediate immersion of the biopsies into cold acetic acid may have prevented this phenomenon.

Mitoses occurring in hair follicles were ignored. Hair follicles made up less than one-eighth of the area surveyed, and the space they occupied was considered as not containing mitoses.