slopes of 150 db/khz were commonly encountered and in one animal the slope was 210 db/khz. The difference between the best frequency and adjacent insensitive portions of the audiometric curve ranged from 28 to 44 db on the low-frequency side and from 27 to 54 db on the high-frequency side (*14*).

Sharply tuned audiograms were encountered only in awake bats. In three anesthetized animals there was some tuning, but it was significantly different from that seen in the same bat after recovery from the anesthesia (Fig. 3).

The CF portion of the pulse was analyzed in several bats to determine the relation between the best frequency of the audiogram and the frequency of the CF pulse component (*15*). In all cases the frequency was significantly below the best frequency. The difference between the two frequencies averaged about 1500 hz.

The 1500-hz difference between the frequency of the CF component of the pulse and the best frequency of the bat's audiogram is equivalent to the amount of Doppler shift expected from the flight speed of *C. parnellii* (*10*). It appears that these bats, like the Rhinolophidae of Europe, emit a CF component to which their ears are relatively insensitive, but as long as there is any relative movement between a bat and its target the CF component of the echo can be Doppler shifted into a more sensitive portion of the hearing range (*4, 8*). In our most sharply tuned preparations the audiograms indicated that the cochlear receptor was up to 44 db more sensitive to Doppler-shifted echoes than to the frequencies of the emitted pulse. This, even without neural sharpening mechanisms (*5, 16*), could permit the system to function efficiently during periods of pulse-echo overlap.

Previous experiments have shown that bats in general, and CF bats in particular, have sharply tuned central auditory systems (*5, 9, 16, 17*). What is remarkable, however, is that the system is so exquisitely tuned as far peripherally as the receptor level. No cochlear microphonic audiogram in any vertebrate has yet been reported which even approaches the degree of frequency specificity in *Chilonycteris*.

The finding that anesthesia profoundly affects tuning at the receptor level was completely unexpected. Changes of this type have not been previously reported and are difficult to explain. However, they require that future experiments on the auditory system of CF bats, and perhaps other vertebrates, be carried out on unanesthetized, awake animals.

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6. The cruising pulses of *C. parnellii* are on the order of 20 to 25 msec in duration with a fundamental frequency of about 30 khz and a prominent second harmonic of approximately 60 khz (*2*). During the first millisecond of emission the frequency (second harmonic) often rises from about 56 to 60 khz and then stays constant (the constant frequency portion of the pulse); during the last 2 msec of emission the frequency sweeps downward to about 45 khz (the terminal FM portion of the pulse).
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14. The tuning was not influenced by the electrode position relative to the cochlear aqueduct. In one experiment, the audiogram was examined as the electrode was lowered through the brain in 0.5-mm increments without any apparent change in tuning. In several bats the electrode missed the aqueduct by 2 or 3 mm, but the audiograms were still sharply tuned. In contrast, the absolute thresholds were markedly affected by electrode placement. In the bats that had poor placements but sharp tuning the best frequency thresholds were 30 to 40 db higher than those that had properly positioned electrodes. Among the bats with good electrode placements, there was no more than ± 5-db difference in those thresholds.
15. Pulses were recorded with a condenser microphone during flight, while the bat was hanging on a wall, and while it was restrained as previously described. The output of the microphone was sent to a modified counter (General Radio, model 1192-B) which yielded a d-c signal directly proportional to the signal period in real time. The output of the pulse could thereby be measured to an accuracy of ± 100 hz.
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Nutritional and Environmental Interactions in the Behavioral Development of the Rat: Long-Term Effects

Abstract. *The behavioral effects of early malnutrition and early environmental isolation were observed in male rats. Dietary and environmental manipulations occurred during the first 7 weeks of life, after which followed a 10-week recovery period. On the basis of several different responses, it was found that the behavioral effects of early malnutrition were exaggerated by the environmental isolation. In most cases, the behavioral effects of early malnutrition were completely eliminated by supplying "additional stimulation" early in life. Two theoretical mechanisms are proposed to explain these findings.*

Malnutrition suffered early in the life of a child (*1*) or an experimental animal (*2*) has been shown to result in long-term behavioral abnormalities. In animals, the most characteristic change in adult behavior is an increase in emotional responsiveness (*3*). Other kinds of manipulations introduced very early in the life of animals also have long-term consequences upon adult behavior. Stimulation produced by daily handling of the neonatal rat has been reported to decrease adult emotionality (*4*). Moreover, early social or environmental isolation has been found to result in an increase in the emotional responsiveness of animals (*5*). The purpose of the study reported here was to compare the effects and possible interactions of early malnutrition and environmental conditions on various aspects of adult behavior in the rat.

All experimental manipulations occurred within the first 7 weeks of life. A 2 × 3 factorial design was used combining two levels of nutrition and three environmental situations. The nutritional treatment was as follows. Pregnant rats gave birth within our laboratory. The pups were reduced to eight per dam. All males were retained, and females were discarded. The dams were then

randomly divided into two groups, one group receiving an optimum diet composed solely of purified ingredients containing 25 percent (by weight) casein and the other group receiving a low-protein diet containing 12 percent casein which caused a reduction in milk production (*6*). The male pups were weaned at 21 days of age. The pups from dams receiving the 25 percent casein diet continued with this diet for the duration of the experiment. The pups from the protein-restricted dams were weaned to a 3 percent casein diet for the next 4 weeks, then were given the 25 percent casein diet.

The control environmental conditions consisted of maintenance of the neonatal animals in standard rat nursing cages for the 3-week preweaning period (18.3 by 23.0 by 13.7 cm, stainless steel, solid-sided boxes). The animals were then moved to individual wire cages where they remained for the balance of the experiment. These animals were handled only once a week for weighing purposes, and their food cups and water bottles were changed every 3 days.

The conditions for environmental stimulation consisted of housing the neonates in the same type of nursing cage as was used in the control conditions, but in these cases the pups were picked up and handled for approximately 3 minutes every day during the nursing period. After weaning at 3 weeks they were housed in wire cages in pairs. They continued to receive daily handling and, in addition, were placed in a large wooden box (58 by 34 cm) containing many different toys along with five other animals of the same group for a 1-hour "play" period 5 days a week.

The conditions for environmental isolation consisted of a minimum of handling during the suckling period. At weaning, the animals were placed individually into wire cages within a closed wooden chamber. Each cage was isolated from the others by means of wooden partitions on all sides. These animals received no handling, and the chamber was opened only every 3 days to provide adequate food and water. The chambers produced a lightproof, sound-attenuated environment. Blowers provided air circulation and a continuous masking noise.

At 7 weeks of age all animals were given the same environment and nutritional conditions as the controls. Each group had no fewer than eight animals.

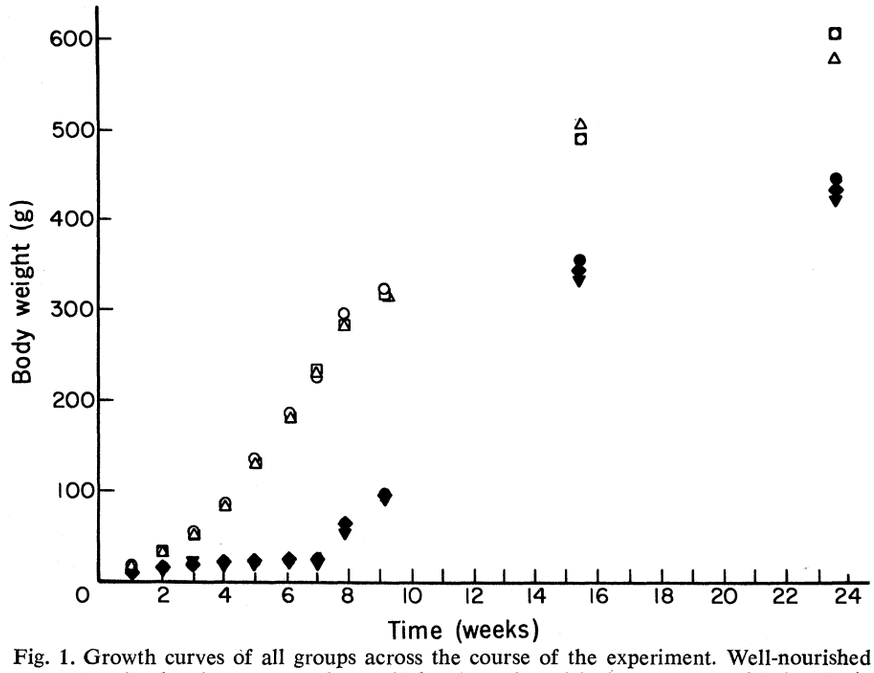

Fig. 1. Growth curves of all groups across the course of the experiment. Well-nourished rats: ○, stimulated; □, control; △, isolated; malnourished rats: ●, stimulated; ♦, control; ▼, isolated.

Ten weeks of recovery were allowed before behavioral testing was begun.

The effect of the low-protein diet was quiet severe, resulting in very little growth (Fig. 1). Recovery was quite rapid, although the growth rate of the previously malnourished rats decreased to the same level as that of the controls at about 15 weeks and remained at a level similar to that of the controls for the duration of the experiment. There was no significant effect of the environmental variables on growth rate.

The first behavioral test consisted of a measurement of the amount of locomotion in an open field. The apparatus consisted of a large wooden box (27.0 by 34.5 by 45.5 cm) with a Plexiglas front. Four photocells, equally spaced across the length of the box, were located 5.5 cm above the floor. A response was electronically counted only when the animal moved completely from one photocell beam to another. This procedure eliminated the possibility of extraneous counts as a result of mere body position such as tail or head movement. Each session lasted 20 minutes. The results are presented in Fig. 2A.

An analysis of variance showed open-field locomotion to be significantly increased by early environmental isolation ($P < .01$) and early malnutrition ($P < .01$). Moreover, the significant interaction ($P < .02$) indicated that early environmental isolation had a greater effect on the rats exposed to early malnourishment than the well-fed controls.

The next series of behavioral observations involved various aspects of social behavior. Two animals of the same group were placed in the same observation box for a 20-minute session. An observer recorded the frequency and the duration of mutual grooming, fighting, and following behavior. Frequency measures were found to be too variable to analyze. The duration data are presented in Fig. 2, B through D. Although the previously malnourished rats showed a higher mean time spent mutually grooming than their well-nourished controls, the effect was not found to be significant.

A following response was defined when the movement of one animal across the field was closely followed by the other animal. The environmental conditions had no significant effect on following responses in the well-nourished groups, but early environmental restriction produced a highly significant effect in the early-malnourished animals ($P < .01$) (Fig. 2B).

A fighting response was defined when the two animals engaged in fighting, which usually was accompanied by some squealing and escape movements by one of the animals. As in the following response and in the locomotor response, the effect of early environmental conditions was considerably more profound in the early-malnourished groups than

in their well-fed controls. The difference in fighting time between the early-stimulated and early-isolated group was statistically significant ($P < .01$), whereas no significant difference could be observed in the well-nourished animals (Fig. 2C).

Finally, the tendency of the animals to explore a new environment was observed. Only the early-stimulated and early-isolated animals were used. A small room (25.5 by 23 cm) was built adjacent to the open field which was connected through a 12- by 12-cm opening. The animals were placed individually in the open field for a 55-minute period. The percentage of the animals of each group which entered the "new room" for every 5-minute segment is plotted in Fig. 2D. Here also the interaction between early malnutrition and environment becomes apparent. There was no effect of early malnutrition in the early-stimulated animals. On the other hand, when early malnutrition was combined with early environmental isolation, a marked reduction in expolaratory behavior resulted. A chi-square test proved the difference significant ($P < .05$). Moreover, no discernible effect of the early isolation could be observed in the well-nourished group.

Frankova (*7*) was the first to suggest that an interaction may exist between early nutrition and early environmental conditions in the development of behavior in animals. Our data are in complete agreement with this view. In all responses observed except the fighting response, whatever effect early malnutrition produced, its effect was always exaggerated by environmental isolation and depressed by environmental stimulation. Levitsky has shown that early malnutrition results in an increase in locomotor activity when tested in a small open field (*8*); this response is positively correlated with other measures of emotionality (*3*). Moreover, decreases in exploratory behavior were also observed to result from early malnutrition (*9*).

The fighting response, however, appears in contradiction. During the 1-hour "play" period of stimulation, where six animals of each dietary group were placed in an enclosed "playground," the malnourished animals were observed to engage in considerably more fighting behavior than their well-nourished controls. In the isolation conditions, on the other hand, there was no occasion for the fighting response to be observed and thus no opportunity for this response sequence to be altered in the malnourished animal.

The mechanism through which early malnutrition and environmental stimulation may interact to produce long-term behavioral changes is not at all clear. There exist at least two possibilities. Malnutrition may change the experience or perception of the environment during the period of early development by physiologically rendering the animal less capable of receiving or integrating, or both, information about the environment. Many profound changes take place within the central nervous system as well as in the rest of the body during a period of malnutrition in early development. Decreases in brain size (*10*), DNA content of the brain (*11*), myelinization (*12*), cortical dendritic growth (*13*), brain cholinesterase content (*14*), and brain norepinephrine control (*15*) have been reported in young malnourished rats. Environmental stimulation produces changes in brain norepinephrine content (*16*) and cholinesterase content (*17*), as well as cortical dendritic growth (*18*). Thus, physiological mechanisms which may be responsible for the long-term effects of early stimulation may not be operative because of a concurrent state of malnutrition during a critical period of development.

Another mechanism through which early malnutrition and environmental variables may interact may be purely behavioral in nature. Malnutrition may produce behavior that is incompatible with the incorporation of environmental information necessary for optimum cognitive growth. In the case of a malnourished animal, the behavior may be primarily food-oriented and, in the case of a malnourished child, the behavior may be expressed as apathy and social withdrawal (*19*). Thus, specific kinds of information or specific behavioral responses which may be required for optimum cognitive development as reflected by test behavior or educational performance may be absent or reduced in the malnourished child as a result of a higher priority of responses elicited by the malnutrition.

The demonstration of a behavioral interaction between early nutritional conditions and the environment of young animals not only demonstrates the

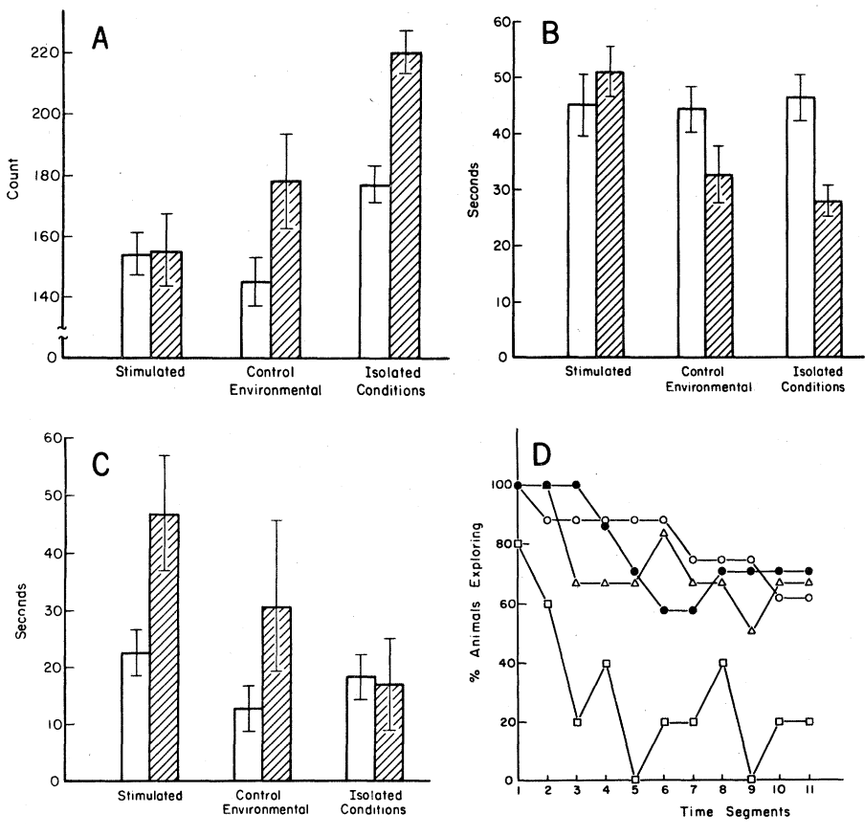

Fig. 2. Mean and standard error of (A) locomotor, (B) following, and (C) fighting responses. (Shaded area) Low-protein diet; (unshaded area) control diet. The exploratory response (D) is expressed as the percentage of animals within each group that entered the new environment per 5-minute time segments during the course of the test session. Well-nourished rats: ●, stimulated; △, isolated; malnourished rats: ○, stimulated; □, isolated.

complexity of understanding determinants of behavior, but also points out the profundity of early experience and early nutrition as major contributors to ultimate adult behavior.

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The Chimpanzee as an Animal Model for Investigating Alcoholism

Abstract. *Young chimpanzees* (Pan troglodytes) *will accept ethanol in quantities sufficient to produce symptoms of withdrawal when ethanol is subsequently discontinued. Mild to severe symptoms of physical dependence, including grand mal seizures, are observed when ethanol is abruptly withdrawn after 6 to 10 weeks of chronic oral intake. In addition, the rate of disappearance of ethanol in blood increased during periods of chronic ingestion, an indication of developing metabolic tolerance. These results suggest that the young chimpanzee may be a suitable model for experimental studies of alcoholism.*

Experimental investigation of the parameters associated with alcoholism has been limited by the lack of suitable animal models. Recently, however, Freund (*1*) reported physical dependence in mice after oral intake of ethanol, and Goldstein and Pal (*2*) obtained withdrawal reactions in mice by forcing them to continuously inhale ethanol while partially blocking ethanol metabolism with daily injections of pyrazole. Physical dependence has also been produced in dogs (*3*) by administration of ethanol through an implanted gastric cannula, and in dogs and rhesus monkeys (*Macaca mulatta*) (*4*) by introduction of aqueous ethanol solutions directly into the stomach, through a gastric catheter. With the exception of Freund (*1*), who reduced body weight to enhance ingestion of a liquid diet containing ethanol, all of these investigators employed forced administration to achieve physical dependence. We have obtained an increasing oral acceptance of ethanol by chimpanzees (*Pan troglodytes*), and have achieved pharmacologically significant concentrations of ethanol in blood and subsequent withdrawal reactions in those subjects. Thus, animal models may be appropriately used in investigations of alcoholism. Specifically, we may use as such a model, one of the species most closely related to man, the chimpanzee.

When chimpanzees were 1 to 7 months old, we offered them doses of ethanol mixed with a liquid diet at scheduled feeding times. Normal weight gains were maintained in these developing animals with a liquid diet based upon a standard nursery formula (SMA, Wyeth) combined with Provimalt (C. B. Fleet Co.), a high protein additive (*5*), and with a multivitamin preparation. Ethanol or isocalorically substituted lactose was then added so that 19 percent of the total daily caloric intake was derived from protein, 16 percent from fat, 20 to 65 percent from carbohydrate, and 0 to 45 percent from ethanol. The animals were offered this mixture in baby bottles four to five times daily, depending upon their age. Since the ethanol dose for each animal is dependent upon his acceptance of the liquid diet, we found it necessary to begin by offering ethanol in concentrations as low as 1 percent (weight to volume). We then gradually increased the concentration to as much as 10 percent, resulting in daily doses of from 2 g/kg of body weight initially to as high as 8 g/kg during the weeks prior to withdrawal. At the higher daily doses (above 5 to 7 g/kg) fluctuating concentrations of ethanol in blood were continuously present, reaching diurnal maximums of 250 to 500 mg per 100 ml of blood. Corresponding diurnal minimums of 50 to 300 mg per 100 ml of blood occurred just before the first morning feeding. Since the animals were not fed between 11 p.m. and 8 a.m., a pattern of withdrawal symptoms began to emerge during the early morning hours if the concentration of ethanol in blood decreased to less than 100 to 150 mg per 100 ml of blood. The observed hyperreflexia, mild tremulousness, and irritability were readily reversed when ethanol was administered in the first morning feeding. Thus, the continual presence of ethanol in the blood appears to be an important factor in the development of physical dependence, an observation consistent with other reports (*1, 6, 7*).

By gradually increasing the dosage, the animals only infrequently refused their ethanol-containing diet. When an occasional refusal did occur, however, the ethanol concentration was temporarily reduced, and a normal level of food consumption was reestablished, so that the possibility that the coercive