the dominant female, did not show the phase of depression. During the agitation phase, unlike the other infants, she spent a great deal of her time with the adult females in the group. As she recovered she became actively involved in exercise play and in exploration of the inanimate environment, followed later in the month by social play. However, the nondepressed infant showed many of the same behavioral changes as the other infants.

During the separation month all four showed a significant increase in self-directed behavior ($P < .05$) and exploration of inanimate objects ($P < .01$), and a significant decrease in play ($P < .01$), both social and nonsocial. The early reaction to separation included a drastic fall in social play and a great rise in self-directed behavior, whereas recovery was accompanied by a gradual rise in social play and normal levels of self-directed behavior (Fig. 1).

When the mother was reintroduced to the group another dramatic change occurred. There was a tremendous reassertion of the dyadic relationship with marked increases in various measures of closeness in all four pairs. Clinging by the infant (Fig. 3), protective enclosure by the mother, and nipple contact all rose significantly ($P < .01$) in the month after the reunion as compared to the frequency of these actions in the month before separation. Even in the third month after the reunion this trend was evident. This significant rise in measures of dyadic closeness is particularly striking in view of the fact that ordinarily for the age periods involved (*8*) these particular behaviors fall considerably.

The increased closeness was manifest in other ways as well. A measure of mother-infant physical separation that we have found valuable in our normative studies concerns departures (usually by the infant) to another level of the pen. The frequency of such departures during the month after the reunion fell to 20 percent of the departures in the month before the separation. Furthermore, the mean duration of these departures fell from 60.5 seconds to 34.4 seconds. Finally, maternal behavior which normally discourages dyadic cohesiveness at this age, such as punitive deterrence and nipple withdrawal, appeared very rarely (*9*).

The individual differences in the reaction to separation may in part be explained in terms of the ontogenetic influence of the regulation of monkey behavior by the dominance hierarchy (*10*). The offspring of dominant females may develop greater coping ability and thus have a greater likelihood of survival if the mother is lost. This is consistent with selective advantage of dominance.

The stages of the reaction appear to be successive efforts at adaptation. The first two stages are comparable to the two basic response systems proposed by Engel (*11*) as available to the organism for dealing with mounting stress. The agitated phase, which appears to coincide with Engel's "flight-fight" response pattern, is likely to effect reunion with mother, if she is available. The second stage is strikingly similar to the syndrome of "anaclitic depression," reported by Spitz (*2*), in human infants separated from their mothers, an example of the response pattern described by Engel as "conservation-withdrawal, [which] involves inactivity . . . and withdrawal from the environment," and which appears to conserve energy and avoid injury. The striking similarity between the early stages of the reaction to separation of pigtail infants and children suggests that the mediating central nervous response systems may be common to both species. The third stage, recovery in the continued absence of the mother, which was not reported in the human infants, may in the monkey infant be attributed to his greater locomotor ability, which enables him to reengage the environment actively on his own.

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4. The characteristics of the pen are detailed in L. A. Rosenblum, I. C. Kaufman, A. J. Stynes, *Animal Behav.* **12**, 338 (1964).
5. I. C. Kaufman and L. A. Rosenblum, *Primates* **7**, 205 (1966). All scores were transformed into percentages of total time of observation to equate for variations in the length of observation. Repeated measures analyses of variance were utilized throughout to assess the statistical significance of the data.
6. Seay and Harlow (*3*) have described an initial "violent protest" in separated rhesus infants followed by a stage "characterized by low activity, little or no play, and occasional crying." Hinde *et al.* (*3*) in their study of separated rhesus infants also noted increased crying and a reduction in play and locomotor behavior, and described "a characteristic hunched posture."
7. The facial expression had an appearance similar to that which Darwin [*The Expression of the Emotions in Man and Animals* (1872) (Philosophical Library, New York, 1955)] described and believed "to be universally and instantly recognized as that of grief."
8. L. A. Rosenblum and I. C. Kaufman, in *Social Interaction Among Primates* (Univ. of Chicago Press, Chicago, in press); I. C. Kaufman and L. A. Rosenblum, in *Determinants of Infant Behavior IV* (Methuen, London, in press).
9. Hinde *et al.* (*3*) reported that after reunion only one pair showed a long-lasting increase in closeness. Seay and Harlow (*3*) found that despite an increase of mother-infant contact on the first reunion day the increase disappeared within 2 weeks. They concluded that "the result of mother-infant separation on the mother-infant relationship of these animals was transient and apparently unimportant." In this regard our results differ dramatically from theirs, since we found a marked and long-lasting intensification of the relationship in all our dyads.
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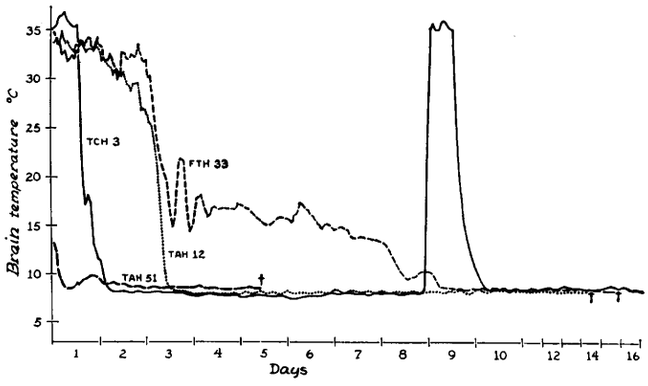
Disruption of Hibernation Caused by Hypothalamic Lesions

Abstract. *Lesions were made in the preoptic-anterior hypothalamic area or ventromedial nucleus of ground squirrels* (Citellus tridecemlineatus). *Four squirrels, two with preoptic damage and two obese hyperphagics, entered hibernation within 1 to 3 days. They all died after 11 to 12 days, shortly after all normal hibernating squirrels had awakened. Seven squirrels with preoptic damage, rendered hypothermic before being placed in the cold, died within 2 to 6 days.*

In hibernators, as in homeotherms, appropriate hypothalamic lesions cause disturbances in three important regulatory systems—temperature, food intake, and sleep and wakefulness. For instance, when ground squirrels were rendered hypothermic and allowed to return to normal body temperature in an environment of 10°C, animals with lesions in the preoptic-anterior hypo-



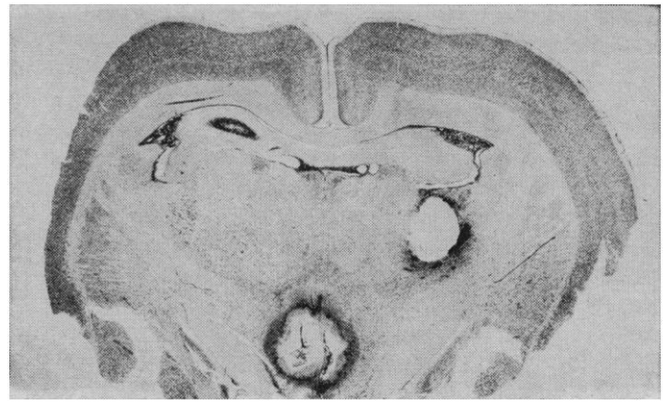


Fig. 1 (left). Course of hibernation and hypothermia in normal and brain-damaged ground squirrels. *TCH 3*, normal squirrel; *TAH 12*, squirrel with anterior hypothalamic lesions; *FTH 33*, obese hyperphagic squirrel; *TAH 51*, squirrel with anterior hypothalamic lesions rendered hypothermic before being placed in the cold. †, Died. Fig. 2 (right). Photomicrograph of brain of squirrel TAH 12 showing the largest extent of lesions in the anterior hypothalamus. Damaged area above and to the right of the lesions was made by the thermocouple tube.

thalamic area took two to six times longer than normal controls to reach a body temperature of 35°C. Squirrels with ventromedial lesions overate and became obese. Squirrels with lateral hypothalamic lesions became adipsic and aphagic and lost up to 35 percent of their preoperative body weight before they began to eat and drink again and returned to their normal weight levels. Posterior hypothalamic damage led to somnolence, mutism, and a slight feeding deficit (*1*).

All these regulations undergo great changes as the hibernator prepares himself for dormancy and finally starts to hibernate. In the summer and early fall, ground squirrels become obese. When they enter hibernation, their temperature drops from their normothermic level (36° to 39°C) to a few degrees above the ambient temperature. Over the entire winter their rhythms of sleep and wakefulness change from a 24-hour cycle to deep hypothermic sleep punctuated by brief arousals every week or so, during which time they are awake and alert, and their body temperature returns to normally high levels.

Lesions which disturb such important regulations should produce aberrations in hibernation which may help us to understand the normal process of hibernation.

Male and female 13-lined ground squirrels (*Citellus tridecemlineatus*) weighing between 170 and 350 g were used. In all animals brain temperature was recorded by implanting in the general area of the thalamus a piece of 18-gauge stainless steel tubing, closed at the tip, and fixing it rigidly to the skull with jewelers' screws and dental acrylic cement. A copper-constantan thermocouple (36-gauge) was introduced into the tube and attached to a Honeywell Electronik 16 multipoint temperature recorder. The experimental group of squirrels was anesthetized with Nembutal (50 mg/kg), and electrolytic lesions were made stereotaxically in the preoptic-anterior hypothalamic area or ventromedial nucleus of the hypothalamus.

When the animals had returned to their preoperative body weight or, in the case of the hyperphagic animals, when they had stabilized their weight (that is, a gain or loss of not more than 10 g per week), they were given sawdust and cotton wadding for nesting material in their individual cages and placed in a cold chamber kept at 6° ± 2°C. The thermocouples were attached to the recorder adjusted to register the brain temperature of each animal once every 15 minutes.

One squirrel, designated as TCH 3 (Fig. 1), is typical of four normal ones that hibernated through the winter. This animal entered hibernation within 2 days of placement in the cold (range 2 to 6 days). He remained at a lowered temperature for 6 days, arose for 15 hours, and then resumed hibernation. This pattern of dormancy punctuated by brief arousals continued all winter in all normal animals. The longest that any normal squirrel hibernated without awakening was 9 days. All these squirrels could be aroused at any time by handling, or by electric shock applied to the hind limb.

Four brain-damaged squirrels entered hibernation within 1 to 3 days in the cold. Two of them had extensive damage in the preoptic-anterior hypothalamus, and two were animals that were hyperphagic and obese (weighing 450 g each from preoperative weights of 170 and 233 g) because of ventromedial lesions. The brain temperatures of these animals fell slowly to 8.0° to 8.5°C which was typical of every animal, normal or brain-damaged, hibernating in this experimental situation. All four built good nests and assumed the normal, tightly curled, hibernating posture. However, none of the brain-damaged squirrels could be aroused by handling, repeated jabbing with hypodermic needles, or electric shock applied to the hind limb. When disturbed, they responded by changing their position in the nest slightly, but they never awoke or showed any rise in brain temperature as a result of outside disturbance. All four died in hibernation without arousing. Three of them died on the 11th day after entering hibernation (squirrel TAH 12 in Fig. 1 is typical). The fourth squirrel, FTH 33 (Fig. 1), arrested his decline in temperature at a level of 15°C for 8 days before reaching 8°C. His temperature began to drop on the third day in the cold. When it reached 15°C, a partial arousal began, and his temperature reached 23°C before declining. This animal died on the 12th day after his temperature reached 15°C.

In order to see if these animals were hibernating or had simply been overcome by the cold and were just hypothermic, a group of seven squirrels with anterior hypothalamic lesions was rendered hypothermic before being placed in the cold. Their brain temperature was brought down to 11° to 13°C by Giaja's technique of hypercapnic hypoxia (*2*), and then they

were placed on nesting material in cages in the hibernating room. Normal squirrels so treated returned to 35°C within 3½ hours. None of the seven brain-damaged squirrels recovered normal temperature, nor could they be aroused at any time by handling or electric shock. They assumed no position other than the one in which they were first placed in the cold. Their brain temperature dropped to 8.0° to 8.5°C and remained there, without arousal, until they died 2 to 6 days later. The temperature curve of squirrel TAH 51 (Fig. 1) is typical of these seven squirrels; the other curves simply stop earlier or later. Two animals with lesions were able to raise their temperature and returned to normal within 4 hours. Histologic examination later showed that the lesions were too low and damaged only the optic chiasm. The seven squirrels (with lesions) that died in hypothermia and the two nonobese squirrels that died during hibernation had large lesions in both the preoptic and anterior hypothalamic areas, including the suprachiasmatic and arcuate nuclei, although the lesions extended more rostrally or caudally in different animals (Fig. 2). The furthest anterior extent of any lesion was at the level of the anterior commissure, and the furthest posterior extent was just anterior to the ventromedial nuclei. The lesions extended laterally to the lateral preoptic nuclei and the fornix, dorsally to just below the paraventricular nuclei, and ventrally to the optic chiasm and base of the brain.

Autopsy revealed that all seven hypothermic squirrels had large ulcers in the stomach or intestinal tract. None of the four squirrels that died in hibernation had any visible ulcers.

The brain-damaged squirrels rendered hypothermic, and the squirrels (with lesions) that entered hibernation normally, remained hypothermic until they died. However, the former died after 2 to 6 days, whereas the latter died after 11 to 12 days. This suggests that the latter group hibernated normally but died because they were unable to arouse. Results from other investigators support this view. Popovic (*3*) has shown that normal ground squirrels (*C. tridecemlineatus* and *C. citellus*) kept hypothermic at a body temperature of 10°C live approximately 110 hours. The brain-damaged hypothermic squirrels in this experiment lived 48 to 140 hours. In our laboratory, a period of 9 to 12 days is the longest that normal ground squirrels (*C. tridecemlineatus* and *C. lateralis*) remain in hibernation before arousing, at temperatures comparable to the 6°C used here. The hypothalamic-damaged squirrels that entered hibernation in this study died after 11 to 12 days.

The necessity for periodic arousals has not previously been proved. Hock (*4*) suggested that arousal takes place when some lethal substance has built up in the animal or when the amount of some nutrient has fallen critically. Thus, if the animal did not wake up, the concentration of the lethal substance would increase (or the amount of the nutrient decrease) to a point where he would be unable to arouse. My research corroborates Hock's view that arousal is necessary for survival and suggests (tentatively, since the number of animals is small) that the timing of the arousals is crucial; the normal animal wakes up just before the unknown, potentially lethal state becomes in fact lethal. The animal does not have much leeway in the time of arousal. If it does not wake up at approximately the outer limit of a normal dormant period, it dies quickly thereafter.

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Phenylketonuria: Enduring Behavioral Deficits in Phenylketonuric Rats

Abstract. *The behavioral deficit in rats made "phenylketonuric" by ingestion of* L-*phenylalanine was evaluated 23 to 65 days following cessation of treatment. Animals treated from birth to 60 days showed significant deficit on reasoning and discrimination learning tests but not on discrimination reversal sets. Animals treated from 30 to 60 days showed significant deficit only on the reasoning test.*

When phenylalanine and related compounds are administered to rats, excess phenylketones are excreted in the urine, and phenylalanine of plasma is elevated. In addition there are frequent reports of deficits in behavior. Although the biochemical reactions are dependable, the occurrence and interpretation of the behavioral deficits are open to question (*1*), making doubtful the adequacy of this procedure as a model of phenylpyruvic oligophrenia.

One question concerns the time of testing of behavior. If the tests are given during or immediately after the administration of phenylalanine, any demonstrated deficit may be attributed to acute effects of this agent. For example, Polidora *et al.* (*2*) demonstrated that the deficit reported for rats treated from time of weaning disappeared after cessation of treatment with L-phenylalanine.

A second question involves the treatment period. Administration of the amino acid during fetal or neonatal life, a period of rapid neural development, is more apt to produce a permanent behavioral deficit, whereas treatment beginning at the time of weaning might not. Results of research with such subjects has been equivocal. Woolley and van der Hoeven (*3*) reported a deficit in mice treated from birth, but not in mice treated from weaning. However, testing occurred before an acceptable period of recovery from the toxic effects. Similarly, the deficit reported by Loo *et al.* (*4*) was measured when the subjects were still receiving phenylalanine.

Perez (*5*) reported no learning deficit in rats treated from birth, but his mortality figure of 70 percent could mask any effect of the amino acid through selective survival. Perry *et al.* (*6*) also reported no deficit, but the treatment periods did not extend beyond 8 days of age, a period too brief to produce permanent neurological or physiological deficit (*7*).

A third question involves the selection of assessment measures. Most tests used to evaluate effects of phenylalanine in rats have involved relatively simple discriminations that frequent-