show (i) that larval *R. catesbeiana* can be made to produce antibodies against their own major frog hemoglobin and (ii) that the hemoglobin of the postmetamorphic animals developed from immunized tadpoles is different from the usual hemoglobin of the adult frog.

There are several possible ways by which the immunization of the tadpoles might have altered the froglet hemoglobin. First, since the animals do not come from an inbred strain, it is conceivable that the only immunized animals able to go through metamorphosis were those with the variant hemoglobin. However, as mentioned above, it seems unlikely that such a variant exists. The presence of antibodies in a developing animal against certain allotypic specificities of that animal may lead to persistent changes of the phenotype of these animals. This has been shown for the rabbit and mouse immunoglobulins (*8*). A similar situation may have occurred in our experiments. The presence in the tadpoles of antibodies directed against their own adult hemoglobin at a time when a new adult erythropoietic cell line was developing (*9*) may have eliminated this adult cell line. Such conditions might favor another cell line which in the normal animal is either present in small numbers or completely absent. These results suggest the potential use of antibodies in altering the course of development by a simple and specific method.

GEORGE M. MANIATIS
LISA A. STEINER
VERNON M. INGRAM
Department of Biology,
Massachusetts Institute of Technology,
Cambridge 02139

References and Notes

1. A. Riggs, *J. Gen. Physiol.* **35**, 23 (1951); L. P. Stratton and E. Frieden, *Nature* **216**, 932 (1967).
2. B. Moss and V. M. Ingram, *J. Mol. Biol.* **32**, 481 (1968).
3. L. P. Stratton and R. W. Wise, *Fed. Proc.* **26**, 808 (1967).
4. G. M. Maniatis, thesis, Massachusetts Institute of Technology (1969).
5. E. L. Cooper, W. Pinkerton, W. H. Hildemann, *Biol. Bull.* **127**, 232 (1964); E. L. Cooper and W. H. Hildemann, *Ann. N.Y. Acad. Sci.* **142**, 647 (1965).
6. N. Gilboa-Garber and D. Nelken, *Nature* **197**, 158 (1963).
7. A. Riggs, B. Sullivan, J. R. Agee, *Proc. Nat. Acad. Sci. U.S.* **51**, 1127 (1964).
8. R. Mage and S. Dray, *J. Immunol.* **95**, 525 (1965); Z. Lummus, J. J. Cebra, R. Mage, *J. Immunol.* **99**, 737 (1967); L. A. Herzenberg, L. A. Herzenberg, R. C. Goodlin, E. C. Rivers, *J. Exp. Med.* **126**, 701 (1967).
9. J. G. Hollyfield, *J. Morphol.* **119**, 1 (1966); W. DeWitt, *J. Mol. Biol.* **32**, 502 (1968).
10. Supported by NSF grant GB-5181 and NIH grant AI-08054.

10 March 1969

Uncus and Amygdala Lesions: Effects on Social Behavior in the Free-Ranging Rhesus Monkey

Abstract. *The effect of uncinectomy on the social behavior of free-ranging rhesus monkeys was observed on Cayo Santiago Island. Operated subjects showed social indifference, failed to display appropriate aggressive and submissive gestures, were expelled from their social group, and eventually died. Two juvenile subjects with the lesion restricted to amygdala survived and have rejoined their social group. Size of lesion and age at operation were major factors in determining the degree of behavioral change.*

While considerable data have accumulated on the behavior of subhuman primates after circumscribed brain lesions, these observations have been limited to the caged animal. In order to extend the scope of our studies of brain behavior to include a long-neglected but vitally important dimension of behavioral observations, we have begun to examine the effects of circumscribed brain lesions on the social behavior of subhuman primates under free-ranging conditions. Several studies of the effects of uncus and amygdala lesions on the social behavior of related species that display different temperaments and social behavior in the cages, as well as under free-ranging conditions, are now under way. In two studies the effects of amygdalectomy on social interaction in groups of laboratory housed macaques have been examined. Rosvold, Mirsky, and Pribram (*1*) have reported that amygdalectomized rhesus monkeys fall in dominance in a group situation but that in a paired situation the operated animal may become dominant. Mirsky (*2*) has noted that after amygdalectomy these monkeys become less competitive for food in a group situation, lose rank in the group hierarchy, and are less fearful of man. The following is a preliminary report on one of our first attempts to study the effects of a brain ablation on behavior in free-ranging monkeys. We chose to begin with observing the effects of amygdaloid lesions on the rhesus monkeys of Cayo Santiago Island (*3*).

The subjects for the study were members of group E of the rhesus colony of Cayo Santiago, a 238-acre (96-hectare) island off the coast of Puerto Rico. Group E and six other social bands (a total population of almost 900 animals) are maintained with Purina Chow dispensed at six hog-feeders located around the island. Within each group, a clearly defined social structure regulates daily life; similarly, a definite rank order characterizes relations between groups. Social status, both within and between groups, determines access to food, space, and, to a degree, reproductive partners. Only a few adult males live in isolation from existing groups. Group cohesion appears to be maintained by familial ties and by patterns of feeding, grooming, and reproduction. Relationships between individuals are expressed and maintained by facial, postural, and vocal communication (*4, 5*). All animals were tattooed on chest and thigh, easily identified, and not too perturbed by human observers after the first few weeks of contact.

In this setting, the 85 members of group E were observed by Dicks from early birth season (March) to early mating season (August) of 1968. The observer attempted to distribute a maximum of 25 hours of observation per week over all members of the group. Patterns of association, grooming, and aggression, and reproductive and feeding behavior were of primary interest.

At the end of 2 months of observation, when the social habits of the subjects began to emerge from the data, two young adult males (5 and 6 years old, hereafter called 5AU and 6AU) were selected for operation and trapped. Here, as in all cases, care was taken to select one animal from intermediate dominance levels of each age group. Amygdaloid nuclei and uncinate cortex were removed bilaterally, aseptically, under Nembutal anesthesia.

After the operation these two animals were kept together in a large enclosure [approximately 20 by 20 feet (6 by 6 m)]. Both remained relatively inactive, sitting in what appeared as a withdrawn state but with their eyes open. However, both responded appropriately to animals threatening through the wire walls of the enclosure. In the only observed violent encounter, 6AU defeated the previously dominant, younger, and smaller 5AU. The younger monkey tended to follow and sit close to the older. Neither began normal feeding until the doors of the food bin were propped open by the observer; before operation, these animals fed only after another monkey had opened the doors.

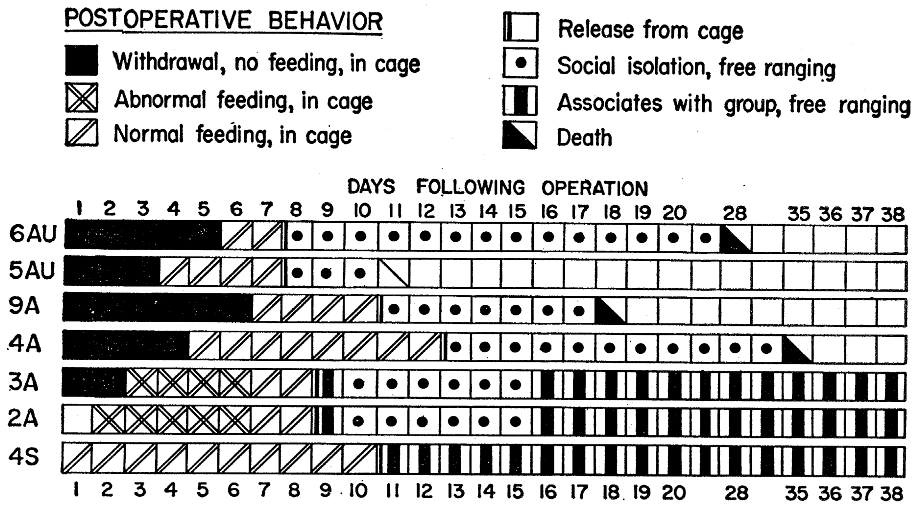

Fig. 1. Chronological diagram of postoperative behavior for six animals that were operated on (6AU, 5AU, 9A, 4A, 3A, and 2A) and one control (4S). AU signifies removal of amygdala plus uncinate cortex, bilaterally; A, removal of amygdala, bilaterally. Numerals signify age of animals in years.

After a recovery period of 7 days, the operated animals were released at a time when the other members of group E were within 100 feet of the enclosure. Curiously, the operated animals showed no interest in their comrades and failed to leave the cage until later forced out by the observers. The two animals failed to return to their own social group but instead ambled forth into the midst of a part of alien group C, a type of behavior never observed normally. Members of group C immediately threatened 5AU and 6AU; 5AU escaped by disappearing in the underbrush while 6AU climbed a tree. A fight broke out between 6AU and a young adult male of group C. After a strong defense 6AU also disappeared into an overgrown area nearby. Neither monkey rejoined group E. The younger animal was seen to avoid approaching members of group E, whereas the older one never returned to areas normally occupied by his comrades. Although both avoided other monkeys, neither reacted to the approach of the observer, even up to within 10 feet. The observer was able to demonstrate that the operated animals could differentiate him from monkeys at a distance of 150 feet in the forest.

Within a few days, both animals displayed large open wounds about the back and limbs. The younger one, 5AU, was cornered by a less dominant group and attacked by at least one less dominant member of his own group (*6*); he died of severe wounds within 4 days of his release. The older animal, 6AU, continued to avoid others for about 3 weeks, although he showed occasional wounds. He was seen only when forced into the sea by others, which suggests that he spent much time in hiding. He was last seen 3 weeks after release and was presumed dead after a further 4 weeks of nonsighting.

In view of these grim results, the extent of ablation was reduced in subsequent operations. Five males (9, 4, 4, 3, and 2 years old, hereafter designated 9A, 4A, 4S, 3A, and 2A) were selected and trapped. Four underwent total amygdalectomy, but sparing the cortex of the uncus; the second 4-year-old, 4S, underwent a sham operation (anesthesia plus incision). One operated animal, 4A, was caged alone; the others, including the sham-operated animal, were observed in a group cage for 1 week both before and after surgery. All animals were released individually after being carried to the center of their home group.

The 2- and 3-year-old operated animals began to eat 2 and 3 days after the operation, but displayed the peculiar eating habits of amygdalectomized monkeys (*7*), that is, tendencies to reach for more chow with a pellet already in hand (especially when distracted) and to hold chow in front of the mouth without chewing. The sham-operated animal began to eat on the first day after surgery; the 9-year-old began 7 days after surgery. The isolated operated animal began eating only after the doors of the food bin were propped open. These last three animals did not show abnormal eating habits.

Within the group cage, the three operated animals and the control showed few significant changes in behavior after operation; 9A exhibited depressed activity and responsiveness to stimuli during the early days postoperatively. Preoperative dominance relations among the four were reestablished, unchanged. All engaged in normal grooming, and all reacted appropriately to threat after the initial period of depressed responsiveness subsided.

Upon release, the sham-operated animal (4S) rapidly joined his mother, then began to threaten and chase his younger brother. This increase in aggressiveness persisted for 2 days, frequently involving other members of the group. In this period 4S also showed submissive gestures (for example, grimaces and perineal presentations to more dominant animals). Over the next 3 weeks (to the end of observations) this animal appeared to behave normally.

Similarly, 2A and 3A each ran to his own mother and enlisted her aid in threatening the experimenter or other animals. Neither displayed clearly submissive gestures toward more dominant animals. After these initial encounters, 2A and 3A drifted away from the group and were not seen over the next week of observation. Then they returned, at the same time, to associate normally with their mothers and other members of the group. By the termination of the observation period these animals were not observed to display grossly apparent irregularities in behavior.

Upon his release, 9A was approached by the leader of group E. The operated animal reacted to this threat by attacking his leader and was promptly chased from the group. Afterward 9A lurked alone on the edge of group E until his death 1 week after release. In addition, 4A left the group immediately after release (in this case, no violent interaction was seen) and moved to a region occupied by another group, before returning to the fringe of his own group. He died about 3 weeks after release. Both 9A and 4A avoided contact with other monkeys but allowed the observer to come within 10 feet. Neither suffered major wounds as a result of attack by other monkeys. Both appeared to weaken and eventually died from starvation, intercurrent infection, or both. It was unfortunate that it was not possible to recover the brains of the operated animals that died; thus no histological verification of the lesions was possible. The corpses of dead animals are quickly devoured by the numerous land crabs inhabiting the island.

In summary, the data suggest that two factors influenced the degree of change in behavior after amygdalectomy—the amount of tissue removed and the age of the operated animal. Total removal of the uncus including amygdala and the cortex of uncus produced the most severe result (in 5AU and 6AU) while removal of amygdala alone along with a small portion of cortex of uncus anteriorly produced a lesser change (in 9A and 4A). The latter type of lesion appeared to produce lesser effects in younger animals (2A and 3A) as seen from Fig. 1.

It is more difficult to specify concisely the change in behavior. The change is toward social indifference. Although operated animals react appropriately when interaction is forced upon them, they appear retarded in their ability to foresee and avoid dangerous confrontations. They do not initiate social activity. They fail to seek out and reestablish their membership in the group. When juxtaposed to their group, these operated animals tend to be attacked and banished as would-be strangers from other groups, perhaps because of inappropriate behavior and responses to approach. As solitary animals, they are vulnerable to attack and unable to compete for food.

In another study (*8*), conducted simultaneously on the effects of total amygdalectomy in free-ranging vervets (*Cercopithecus aethiops*), a somewhat similar result was obtained in that all operated subjects withdrew from social interactions, would not respond to solicitous behavior by normal group members, and none of the operated animals were observed to rejoin a social group.

DENNIS DICKS
RONALD E. MYERS
ARTHUR KLING

Department of Psychiatry, University of Illinois College of Medicine, Chicago, and *National Institute of Neurological Diseases and Stroke, San Juan, Puerto Rico*

References and Notes

1. H. E. Rosvold, A. F. Mirsky, K. H. Pribram, *J. Comp. Physiol. Psychol.* **47**, 173 (1954).
2. A. F. Mirsky, *Ann. New York Acad. Sci.* **85**, 785 (1966).
3. C. B. Koford, in *Primate Behavior*, I. DeVore, Ed. (Holt, Rinehart & Winston, New York, 1965), p. 160.
4. S. A. Altman, *Ann. New York Acad. Sci.* **102**, 338 (1962).
5. D. S. Sade, thesis, Harvard Univ. (1966).
6. J. Loy, personal communication.
7. A. Kling, *Psychosom. Med.* **28**, 155 (1966).
8. ———, J. Lancaster, J. Benitone, in preparation.
9. Supported by the National Institute of Mental Health interdisciplinary training program USPHS MH 8396 and USPHS HD 02277.

16 April 1969

■

Origin of Repopulating Cells after Localized Bone Marrow Depletion

Abstract. *The restoration of marrow in a mechanically depleted segment of rabbit femur is locally determined and apparently initiated by cells normally resident in bone. This conclusion follows from results of two types of radiation experiments: local x-irradiation of the femur shaft immediately before depopulation and x-irradiation of the total body with exception of the femur diaphysis which was depopulated either just before or after irradiation. In contrast to the rapid initiation of marrow restoration in an unirradiated femur, there was little regeneration during the first 3 weeks in an irradiated femur. Recovery of the shielded depopulated femur in the rabbit that otherwise received total-body irradiation was similar to that in the unirradiated animal. Hence, it would appear that the seeding of circulating hemic stem cells is not essential to repopulation and that cells with hematopoietic potential are present in osseous tissue.*

When marrow is removed from a femur shaft either surgically (*1*) or by perfusion (*2*), trabecular bone encroaches temporarily on the evacuated space and the onset of obvious marrow restoration coincides with revascularization of the cavity and resorption of the invading trabeculae, the entire regenerative process being completed within a matter of several weeks. The stimulus for such regeneration appears to be locally determined, since the tissue that is removed represents only a small percentage of the total marrow cells and does not result in any significant perturbation of the number of peripheral blood cells. Although it is possible that repopulation is accomplished by a few cells that may have been left behind in the shaft, microscopic examination of the endosteal surface immediately after marrow expulsion by dextran perfusion has failed to disclose adherent cells (*2*). Autoradiographic analysis of marrow imprints after tritiated thymidine (^{3}HTdR) labeling also reveals that repopulation is apparently not initiated by a growing edge from the epiphyseal marrow (*2*). The studies reported here indicate that seeding of circulating stem-type cells is not essential for repopulation of an evacuated femur segment. Rather, the results suggest that the regenerative process may be initiated by cells normally resident in bone.

New Zealand white female rabbits, weighing 2 to 2.5 kg, were used as the experimental animal, and x-irradiation was employed to determine the local or systemic origin of the repopulating cells. Bone marrow was removed from a 3-cm segment of the right femur by perfusion with a dextran solution in a manner described previously (*2*). In brief, marrow removal was accomplished by perfusion and simultaneous aspiration through Turkel needles inserted into the proximal and distal ends of the femur diaphysis of the test animal under sodium pentobarbital anesthesia. Two types of radiation experiments were performed: (i) local x-irradiation of the femur shaft with 1000 r immediately before depopulation; (ii) x-irradiation (800 r) of the total body, with the exception of the femur shaft that was depopulated either just before or just after irradiation. The x-ray source was a Maxitron (General Electric) operated at a peak of 300 kv, 20 ma, half-value layer 1.65 Cu. For irradiation of the femur shaft only, the target surface distance (TSD) was 30 cm and the dose rate 360 r/min, the rest of the body being protected by a 3-mm lead shield. When the total body, except for a segment of the right femur shaft, was irradiated, the TSD was 120 cm and the dose rate 22 r/min. In this instance, the femur was shielded with 6 mm of lead; the length of shielded shaft was somewhat less (2.7 cm) than that of the depopulated area (3 cm) to minimize the possibility of sparing adjacent normal marrow. The effectiveness of lead shielding was verified by appropriate placement of lithium fluoride dosimeters.

Marrow restoration was evaluated by determining the incorporation of ^{3}HTdR into DNA and the number of cells per unit weight of marrow in conjunction with differential counts. In each treatment group two to six experimental and two control rabbits were killed at frequent intervals. One hour before the rabbits were killed they were injected intravenously with 0.5 microcurie per gram of ^{3}HTdR (specific activity, 11