other thyronines described in this report. It has remained essentially unchanged after more than 1 year of cell culture. These cells also continue to elaborate growth hormone (*18*).

This GH_1 somatotrophic cell system is the first in vitro system which responds significantly to T3 and T4 at physiological concentrations determined in man and other species, as well as to concentrations which have been observed in thyrotoxic man (*1, 7, 17*).

Pituitary somatotrophs also appear in vivo to be a sensitive target cell for thyroid hormone (*19*). The somatotrophic pituitary population decreases in vivo from 40 percent to less than 5 percent of the total cell population in a period of 2 to 3 weeks after thyroidectomy (*20*). Therefore, the thyroid hormone regulation of growth and metabolism of GH_1 cells likely reflects its action on pituitary somatotrophs in vivo.

On the basis of the dose-response relationships for T3 and T4 and other iodothyronines, the GH_1 cell system, as described, appears to reflect certain of the biological actions of the thyroid hormones in vivo and has promise as a model system for study of the regulatory effects of physiological concentrations of thyroid hormone on mammalian cells.

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Behavior of Free-Ranging Macaques after Intraventricular 6-Hydroxydopamine

Abstract. *Macaques* (Macaca mulatta) *observed in a free-ranging colony on Guayacan Island, Puerto Rico, were significantly different in their social interactions, initiatives, facial expressions, and postures after intraventricular 6-hydroxydopamine compared with sham-treated and field controls. This study extends the known effects of 6-hydroxydopamine and catecholamine depletion to the social interactions of a higher primate species under free-ranging conditions.*

We recently reported that intraventricular 6-hydroxydopamine (2,4, 5 - trihydroxyphenethylaminehydrobromide) produced decreases in important positive social behaviors of *Macaca speciosa* observed in an artificially composed social group in a laboratory enclosure (*1*). The changes in social behavior and reductions in brain catecholamines persisted until the animals were killed 16 days after treatment. This suggested that it might be possible to study the effects of central catecholamine depletion on the normal behavioral repertoire, including familial and social relationships, which could be observed most naturally in free-ranging individuals. We now report the first such study of the effects of a specific brain biochemical lesion in free-ranging primates.

Six adult females and two adult males were trapped from two existing colonies of free-ranging macaques (*M. mulatta*) living on Guayacan Island. The females had been living in one social group of about 35 individuals for many years. Four had infants that remained in the group during the captivity period (9 to 21 days). Two large males of identical weight who appeared to be dominant and central animals in their respective social groups were trapped and paired experimentally. The females were paired on the basis of known or probable family relationships or by frequent social interactions or social position in the groups (*2*). The animals, weighing 7 to 12 kg, were implanted with permanent cannulas placed stereotaxically in the lateral ventricle (*1*). After recovery from surgery each pair of awake animals was injected intraventricularly with 6-hydroxydopamine or the carrier (*3*) as follows: 2 mg on the first day, then 4 mg, 8 mg, 8 mg, and 8 mg at 12-hour intervals for a total of 30 mg. On a similar schedule 31 mg produced whole brain norepinephrine depletions of 69 percent in the laboratory (*1*). The animals were observed for adequate oral intake and general condition. On the fourth day after the final injection all monkeys were released across the 80-acre (~ 32-hectare) island from their usual territory. They were followed and observed for an average of 5 hours daily for 10 days from their release. An experienced observer who was blind to their treatment condition recorded the total time during which each individual could be seen, and the frequency or duration of major social interactions (social grooming, self-grooming, threats, attacks, sexual presentations, copulation) and responses to social interactions (*4*). In addition to the four treated and four sham-treated animals, three animals who were not trapped served as field controls.

The monkeys tolerated the cannula

implantations and the injections without serious difficulty. No seizures, vomiting, adipsia, or aphagia were noted. The animals treated with 6-hydroxydopamine assumed energy-conserving postures and had blank, affectless faces, while the sham-treated animals appeared to be unaffected by the carrier solution. Some mild weakness was noted in the treated animals, but in general they appeared less ill or toxic than the animals treated previously in the laboratory (*1*). Although there was evidence that the treated animals had a decrease in appetite during treatment, they were eating and drinking at the time they were released. By that time all ambulated without difficulty and appeared healthy and physically able to survive in the field. All were individually caged during the treatment period, so that no social behavior was observed until the time of release.

Upon release, the treated monkeys were slower to leave their cages than the sham-treated controls, who raced away. Three of the sham-treated animals had traveled back to their infants and families within 15 minutes of the last release, and the fourth returned several hours later. During subsequent observations all of the sham-treated monkeys remained with their original social group and appeared entirely normal; their social activities were statistically and qualitatively indistinguishable from those of the control animals who had remained in the field (see Table 1).

Two of the monkeys treated with 6-hydroxydopamine failed initially to return to their social group, and were seen once on the second day after release in the midst of a social group to which they did not belong. One of the treated females seen there again alone was driven into the sea by males of that social group. She did not drown, however, and was observed again the following day. Neither of these animals appeared to be perceptually disoriented or physically debilitated during this period. After several days all the treated females had returned to their original social group. They were nonetheless seen significantly less often than the sham-treated controls throughout these observations ($P < .05$), which means that they were not with their social group, or were frequently peripheral to it and out of sight.

Quantitatively important specific social behaviors had decreased for the treated group compared to the sham-treated and field controls per total observation time for each animal. The treated monkeys were attacked when they first returned to the group (5.5 ± 2.7 times), whereas the sham-treated controls were not, and the treated animals initiated far fewer threats or attacks than the controls. The treated animals also engaged in less social grooming and self-grooming and in fewer social initiatives (*5*) than the control animals (see Table 1).

The difference in the initiation and reception of aggressive activity between the treated and sham-treated animals is consistent with the fact that the treated females, with the exception of one low-ranking field control, were the lowest ranking of all the adult animals observed. This finding was unexpected on the basis of the previous social and familial pairing, but the absence of definitive data on social ranking before treatment dictates caution in assessing the significance of changes in the social rank of the females. The sham-treated male returned to being a well-integrated but lower-ranking male in his original social group, while the treated male, after being seen occasionally with his original social group, eventually became a solitary peripheral male. Both of these changes sometimes occur in males merely after capture and temporary removal from their social group (*6*). However, the difference in social rank would not explain the large differences between the groups in the total time observed and the percentage of that time spent in positive behaviors.

Although the treated animals appeared sufficiently different for the experimentally blind observer to identify them correctly at the conclusion of the observation period, the change in their physical appearance was subtle. They frequently assumed a slumped posture and would look off into space for several minutes at a time with expressionless faces, but they did not appear sedated. They walked with a particularly deliberate and stiff-legged gait. All but one of the treated animals appeared frightened of the observer and would avoid eye contact more than the other animals. The one monkey who was unconcerned about the observer also became more inappropriate in her responses to the ranks and social signals of other monkeys. Her physical appearance and personal grooming steadily deteriorated during

Table 1. Positive and agonistic social behaviors and a measure of social initiatives (*5*) of free-ranging monkeys treated with 6-hydroxydopamine (6-OHDA) compared to sham-treated and field control monkeys. The two 5-day periods were 4 to 14 days after treatment. Social grooming, self-grooming and total social initiatives were recorded as 30-second units and are reported here as percentages of the time observed. The *P* values are the probabilities of a significant difference between a particular mean and the mean for animals treated with 6-OHDA; SEM, standard error of the mean. Student's *t*-test and Welch's correction for heterogeneous variances were used. Sham-treated and field control animals were not significantly different from each other for any observed behavior.

Animals	Time observed (30-sec units)		*P*	Social grooming (%)		*P*	Self-grooming (%)		*P*	Threats (number per animal)		*P*	Attacks (number per animal)		*P*	Total social initiatives (%)		*P*
	Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM	
Day 1 to day 5																		
6-OHDA treated ($N = 4$)	167.5	39.2		13.9	2.6		2.3	0.8		3.2	1.7		0.5	0.4		17.3	2.3	
Sham treated ($N = 4$)	298.7	34.4	$< .025$	27.2	3.4	$< .025$	4.9	0.7	$< .025$	30.0	10.5	$< .05$	5.5	2.7	$< .10$	34.3	3.6	$< .005$
Field control ($N = 3$)	274.4	36.6	$< .05$	26.8	2.5	$< .01$	3.9	0.8	$< .10$	19.3	9.7	$< .10$	3.6	2.9	$< .20$	32.6	2.9	$< .005$
Day 6 to day 10																		
6-OHDA treated ($N = 4$)	186.6	41.3		15.2	3.3		2.7	1.0		2.3	1.2		0.0			18.3	3.4	
Sham treated ($N = 4$)	312.6	40.0	$< .05$	26.1	3.8	$< .05$	7.3	0.9	$< .01$	32.5	10.7	$< .025$	9.8	3.6	$< .05$	36.6	3.7	$< .005$
Field control ($N = 3$)	267.4	42.9	$< .20$	25.2	3.9	$< .05$	5.5	2.1	$< .15$	7.0	3.1	$< .15$	0.7	0.5	$< .15$	31.2	3.5	$< .02$

the period of these observations. In spite of these changes, however, all the animals were still alive and appeared to be functioning enough to ensure their continued survival at the time they were last observed (March 1973) (*7*).

In general, the observations of free-ranging *M. mulatta* treated with 6-hydroxydopamine confirmed and extended our earlier findings in the laboratory with *M. speciosa* over the same period of time. This study also adds data in the area of social behaviors, appearance, and social relationships not observed or not reported in studies with lower species (*8*). The field situation made it possible to observe more natural behaviors as well as the effects of treatment on stable family and social relationships. Of special interest were two females who failed initially to return to their social group and infants, and one male who returned only briefly. This failure is not considered to be due to the capture, captivity period, anesthetic and tranquilizing agents, cannula insertion procedure, injection procedures, or changes in physical appearance and body image (*6*) since these appear to have been tolerated without exception by the sham-treated animals, who returned immediately to their social group.

The treated monkeys in this study seemed to be healthier than the monkeys in our earlier laboratory study, although a wider range of social behaviors was observed and the effects on them were greater. The appearance of the monkeys was similar in both experiments, especially the blank, affectless faces staring into space. In addition, the changes in social behavior were qualitatively similar to those reported for caged *M. speciosa* after brain catecholamine depletion produced by the tyrosine hydroxylase inhibitor alpha-methyl-*p*-tyrosine (*9*). Changes similar to those reported here were also noted in a field study of the effects of surgical ablation of the amygdala (*10*), including some monkeys being driven into the sea. A catecholamine deficit might be the explanation for the changes observed in all these studies (*11*).

Considerable evidence links catecholamines to the regulation of behaviors mediated by reward and punishment (*12*). Damage to a "reward-punishment system" in the study reported here might be inferred from the decreased engagement in positively reinforced or rewarded behaviors (for example, grooming), and from the failure of some animals to avoid "punishment" (for example, the animal being driven into the sea, or animals failing to avoid attacks by controls). Monoamine mechanisms have also been implicated in studies of mood changes in humans (*13*). Such mood changes might lead to decreased positive social behaviors and social rank, and would also be consistent with the changed physical appearance and the initial failure of some treated monkeys to return to their social group and family. The changes in the appearance and social behaviors of these animals therefore may be relevant to hypotheses relating catecholamines to several human neuropsychopathological processes (*14*). This study suggests that the complex and highly evolved social behaviors displayed by free-ranging nonhuman primates may be useful to further understanding the control of behavior by the brain and its biochemistry. Further observations of these animals are being made to study the long-term effects of 6-hydroxydopamine on nonhuman primate behavior in the presence of natural social and environmental stresses.

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