antiserum was found sufficient to "block" exogenously applied NGF at concentrations containing one biological unit of activity per milliliter. Consequently, the use of undiluted antisera in these experiments was deemed sufficient to block endogenous levels of NGF, which are known to be in the nanogram range (*11*). As a control, samples of whole serum from the same rabbits that were later used to produce the anti-NGF were given to rats as described above. Axon numbers in these rats were the same as in normals (*8*).

After 28 days the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg) and perfused with 0.9 perent NaCl containing 0.2 ml of 1.0 percent $NaNO_2$ and 200 U of heparin per 100 cm^3. When the effluent from the right auricle was free of blood, the perfusion fluid was changed to 3 percent glutaraldehyde, 3 percent paraformaldehyde, and 0.1 percent picric acid in 0.1*M* cacodylate buffer (*p*H 7.4). After 1 to 3 hours, the dorsal roots were removed and placed in the aldehyde solution overnight. The following day the tissue was placed in a solution of 1 percent osmic acid and 1.5 percent potassium ferricyanide (*12*) in 0.1*M* cacodylate buffer (*p*H 7.4) for 1 to 2 hours. The tissue was then stained in 0.5 percent uranyl acetate and embedded in a mixture of Epon and araldite. Thin sections were cut with a diamond knife, placed on single-hole grids covered with Formvar film, stained with 0.1 percent lead citrate or left unstained, and examined in a Philips 300 or 301 electron microscope (Fig. 1). All myelinated and unmyelinated axons were counted. Error in repeated counts of the tissue was ±3 percent. Statistical significance ($P < 0.05$) was determined by Student's *t*-test.

Counts of axons in dorsal roots of the fifth thoracic spinal segment (T5) of untreated rats and anti-NGF–treated littermates are presented in Table 1. There were, on average, 1358 myelinated and 4097 unmyelinated axons in the roots from untreated rats and 1595 myelinated and 5944 unmyelinated axons in the same roots from treated rats. Thus, there were 17 percent more myelinated and 45 percent more unmyelinated axons in the roots from treated animals. These differences are significant at $P < 0.017$ and $P < 0.0001$, respectively.

To our knowledge, this is the first demonstration of a postnatal change in the number of dorsal root axons caused by manipulation of NGF only. The observation that axonal numbers increase after anti-NGF treatment is surprising, because it was previously reported that the number of cells or processes increases when NGF is present and decreases when NGF is not present or is inactivated (*2, 5, 6*). As an explanation, we suggest that NGF acts as a signal from a target cell to the neuron indicating that normal connections are being maintained (*3*). Thus, withdrawal of NGF might indicate that axonal connections are not present, which leads to compensatory sprouting (*4, 9, 13*). Since the axons in the dorsal root are the structural basis of dermatomes, studies are needed to determine whether the qualities of sensation and the size of dermatomes change as a result of such treatment. We also need to determine whether these changes occur in other spinal segments and in other mammals and whether they still occur if the anti-NGF is administered earlier or later in development. Our findings raise the possibility of increasing the number of sensory axons by a noninvasive postnatal technique.

Table 1. Counts of myelinated and unmyelinated axons from rats treated with anti-NGF and from untreated littermates. The counts were made from the left and right dorsal roots of the fifth thoracic segment.

Untreated rats		Anti-NGF–treated rats	
Myelinated axons	Unmyelinated axons	Myelinated axons	Unmyelinated axons
Left dorsal root			
1236	4276	1660	6053
1263	3364	1319	5343
1520	4135	1496	6868
1271	4066	1928	6073
Right dorsal root			
1371	3574	1678	5622
1439	4643	1794	6498
1381	4295	1624	5979
1379	4423	1260	5116
Mean ± standard error			
1358 ±34	4097 ±152	1595 ±80	5944 ±204

C. E. HULSEBOSCH*
R. E. COGGESHALL
J. R. PEREZ-POLO
Marine Biomedical Institute and *Departments of Human Biological Chemistry and Genetics, Anatomy and Physiology,* and *Biophysics, University of Texas Medical Branch, Galveston 77550*

References and Notes

1. L. A. Greene and E. M. Shooter, *Annu. Rev. Neurosci.* **3**, 353 (1980); G. P. Harper and H. Thoenen, *Annu. Rev. Pharmacol. Toxicol.* **21**, 205 (1981).
2. R. Levi-Montalcini, *Annu. Rev. Neurosci.* **5**, 341 (1982).
3. H. Thoenen, Y.-A. Barde, D. Edgar, *Adv. Biochem. Psychopharmacol.* **28**, 263 (1981).
4. J. R. Perez-Polo and B. Haber, in *The Clinical Neurosciences*, vol. 5, *Neurology*, R. N. Rosenberg, Ed. (Churchill Livingstone, New York, 1983), p. V37.
5. E. M. Johnson, Jr., P. D. Gorin, L. D. Brandeis, J. Pearson, *Science* **210**, 916 (1980).
6. H. I. Kornblum and E. M. Johnson, Jr., *Brain Res.* **234**, 41 (1982).
7. C.-B. Jenq, C. E. Hulsebosch, R. E. Coggeshall, J. R. Perez-Polo, *ibid.* **299**, 9 (1984).
8. C. E. Hulsebosch, J. R. Perez-Polo, R. E. Coggeshall, *Neurosci. Lett.* **44**, 19 (1984).
9. ———, *Brain Res.*, in press.
10. C. E. Beck and J. R. Perez-Polo, *J. Neurosci. Res.* **8**, 137 (1982).
11. J. R. Perez-Polo, C. E. Beck, M. Blum, in *Perspectives in Schizophrenia Research*, C. Baxter and L. Melnechuk, Eds. (Raven, New York, 1980), p. 237.
12. L. A. Langford and R. E. Coggeshall, *Anat. Rec.* **197**, 297 (1980).
13. I. A. Hendry, R. E. Bonyhady, C. E. Hill, in *Nervous System Regeneration*, B. Haber *et al.*, Eds. (Liss, New York, 1982), p. 263.
14. Supported by biomedical research support grants S07-RR05427 and S07-RR07205 (C.E.H.), Moody Foundation grants 82-236 (J.R.P.-P.) and 82-231 (R.E.C.), National Institute of Neurological and Communicative Disorders and Stroke grant NS 18707 (J.R.P.-P.), and National Institutes of Health grant NS 17039 (R.E.C.).
* To whom correspondence should be addressed.

19 March 1984; accepted 16 May 1984

Mating in Bighorn Sheep: Multiple Creative Male Strategies

Abstract. *Rocky Mountain bighorn rams obtained copulations by defending single estrous ewes (tending), fighting tending rams for temporary access to defended ewes (coursing), or moving and holding ewes away from other rams beyond the periphery of a traditional tending area (blocking). Coursing and blocking illustrate a feature of many male alternative mating strategies: the ability of males regularly to create mating opportunities.*

A primary and one or more alternative strategies are typically distinguished when different male mating strategies coexist within populations (*1*). Males that use primary strategies generally "create" mating opportunities with some form of sustained intrasexual aggression, of which territorial and harem defense are examples. In contrast, males that use diverse alternative strategies are often depicted as "opportunists," taking advantage of mating chances provided by various extrinsic causes, such as a territorial dispute that leaves females unguarded (*1*). I now present evidence that Rocky Mountain bighorn rams (*Ovis canadensis canadensis*) mate in three distinct ways. A primary strategy, tending, coexists with two alternatives, blocking and coursing. Most males both

block and course, and a few males use all three strategies, within a rut. Blocking and coursing appear unusual compared to other alternative strategies in that males commonly create mating opportunities. This is achieved in blocking by physical manipulation of females and in coursing by physical manipulation of females and tending rams. Although mating interactions have been described (*2*) in which tending and coursing behavior may be discerned (*3*), multiple mating tactics are not recognized generally for bighorns. Here I explicitly distinguish tending and coursing as reproductive strategies and add new information. Blocking of estrous ewes has not to my knowledge been reported previously.

The data were collected from 1979 to 1983 at the National Bison Range in northwestern Montana. All adults in the resident herd of approximately 55 sheep were individually recognizable. I censused the rutting area, usually daily, between mid-November and January each year and watched focal estrous ewes for 193 hours.

In tending, rams consorted with single, estrous ewes and attempted to prevent the approach of other males by threat, body shielding, and physical attack. Only rams in the first third of the male dominance hierarchy tended (*4*). Estrus, the period of ewe receptivity to copulation that preceded abandonment by a defending ram, lasted from 1 to 3 days. Ovulation probably occurs just before the end of estrus (*5*). Tending copulations were frequent [0.90 ± 0.11 copulations per hour (mean ± standard error of the mean), $n = 21$ estruses; 136.2 tending hours] and exceedingly brief (2.3 ± 0.12 seconds, $n = 50$). Nonretaliatory copulations were preceded by extensive courtship, and ewes, although mildly evasive during courtship, stood for copulation.

Tending was highly clumped in space. Eighty-one percent of all census observations of tending ($n = 122$) occurred in only 13 percent or 2.04 km^2 of the ewe group range (Fig. 1A). This concentration apparently resulted from site selection by ewes, since the tending ram did not attempt to control ewe movement. Geographical centers of tending for each year coincided, indicating traditional use of this area by tended ewes.

Tending pairs were often joined by rams of lower dominance rank, called coursing rams, that periodically engaged tending rams in special combat to gain transient access to ewes. Coursing rams instigated combat by approaching a tended ewe. If the tending ram lost balance or defensive position in the ensuing exchange of clashes, butts, and shoves, the coursing ram would run past the tending ram to the ewe. Tending rams bypassed in this way often attempted to defend further by butting the coursing ram on the rump and flank. Coursing rams that breached tending ram defense pursued the ewe and attempted to copulate before the tending ram could recover. Mounts were counted as (apparent) copulations when coursing rams made thrusting motions while firmly pressed against the ewe. Although coursing rams often defended their position in a chase when two or more participated simultaneously, they did not defend tended ewes outside of chases. These males often watched but did not intervene in chases involving subordinate coursing rams or left tending pairs after copulating.

Coursing ram copulations were not preceded by courtship, and ewes appeared not to cooperate: ewes ran or walked as the coursing ram mounted in 94 percent of 84 copulations. Individual coursing males averaged 0.39 ± 0.06 copulations per hour of association with tending pairs ($n = 13$ rams, 209.5 hours of association). Ewes experienced an average of 0.80 ± 0.16 coursing male copulations per hour tended ($n = 21$ estruses, 136.2 tending hours), or nearly half of all copulations observed for tended ewes. The numerous tending and coursing male copulations were intermixed throughout the period of estrus (1 to 3 days). Tending rams never succeeded in completely preventing coursing ram copulations.

Although the fertility of coursing versus tending ram copulations cannot be determined by field observation, the following evidence indicates that rams do reproduce by coursing.

1) Tending rams responded to coursing ram copulations with retaliatory copulations, which suggests regular transfer of sperm by the latter. Seventy-nine percent of 47 sequences of coursing male copulations were followed within 10 minutes by a tending male copulation. In these intervals, tending ram copulation rates exceeded that expected if tending rams scheduled copulations independent of coursing ram copulations ($P < 0.001$, two-tailed binomial test). Occasional failure to retaliate may have resulted from tending ram sperm depletion; their copulation rates were higher in the hour preceding unretaliated versus retaliated copulations ($P < 0.01$, $U = 132.5$, two-tailed Wilcoxon two-sample test).

2) Bighorn testes are large (Fig. 2). Among primates, testes are large relative to body weight in species whose mating systems favor multiple insemination of females by different males (*6*). Similarly, large testes size in bighorns may be an evolutionary consequence of a numerical sperm competition generated among rams by frequent transfer of sperm in coursing.

At other times, individual rams engaged in blocking behavior; that is, they attempted to prevent one or more ewes

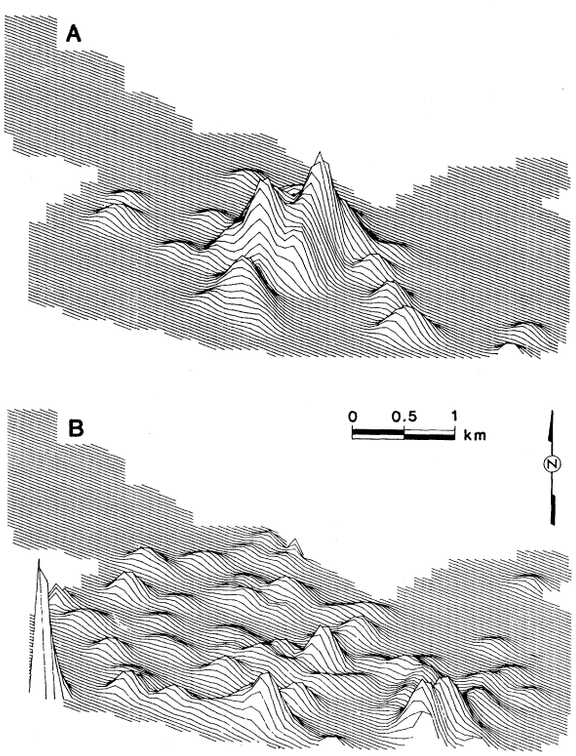

Fig. 1. Distribution of (A) tending and (B) blocking relative to the ewe group range (hatched area). Elevations estimate probability density surfaces.

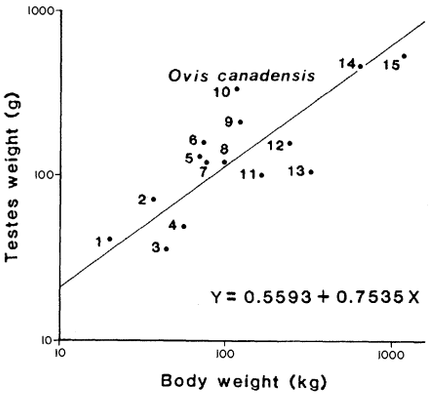

Fig. 2. Log of body weight (kilograms) plotted against log of combined testes weight (grams) for 15 genera of polygynous, pecoran ruminants. Numbers index genera [see (*12*)]. Points are means of individual weights. Fitted line is the reduced major axis. No heterogeneity in slope between the cervidae and bovidae was detected. Dates of body weights for genera 2, 5, and 12 were not given; otherwise all weights are for adults during the breeding season. Testes and body weights are from the same individuals in ten genera and from different individuals in the same geographical region in five genera (2, 5, 12, 13, 15). The deviation (perpendicular to the abscissa) of bighorn testes weight from the regression line is extreme relative to the distribution of deviations for the other genera [$P < 0.03$, $t = 2.14$, one-tailed t-test (*13*)].

from traveling in particular directions. Attempts by a ewe to escape the ram were blocked by position, threat, or physical attack. Even 2-year-old rams exceeded ewes in horn and body size, and successful blocking apparently depended on males using these asymmetries to increase sufficiently the risk of escape. Although the rams prevented ewe movement over a range of directions approximately equal to 180 degrees, they assumed positions relative to stationary ewes that showed this arc of forbidden directions to be centered on a line connecting the ewe to the center of all tending (Fig. 3). A blocking ram often encouraged ewe movement away from the tending area by threat or physical attack. Thus, blocking rams moved and sequestered ewes beyond the periphery of the traditional tending area, thereby placing them in terrain that fewer rams searched. Blocking locations were more dispersed and less predictable than tending locations (Fig. 1B).

Although blocking often involved anestrous ewes, particularly when groups of ewes were blocked, rams preferentially selected near-estrous ewes at the point of focusing on single ewes [$P < 0.001$, $\chi^2(11) = 97.3$] (Fig. 4), and 35 percent of blocking episodes with known outcome ($n = 100$) eventually included all or part of the estrus of one or, rarely, two ewes. In one extreme case, a ram sequestered a ewe for 9 days, including the 7 days preceding her 2-day estrus. Blocking pairs involving estrous ewes resembled tending pairs in form and duration of courtship, ewe reaction to ram mounting, and frequency of copulation (0.83 ± 0.07 copulations per hour, $n = 10$ estruses; 56.5 hours). Coursing sometimes occurred if such pairs were discovered by subordinate rams.

Overall success in blocking varied among the years of the study. However, even in the least successful year (1980), 18 percent of all census observations of estrous ewes ($n = 33$) involved blocking, and the remainder involved tending. Because it was difficult to find blocking pairs, ewes not found during estrus in 1980 (25 percent) were likely to have included a disproportionate number that were blocked.

Alternatives resembling coursing are apparently rare among ungulates. In bighorns, the evolution of coursing probably depended upon the prior existence of (i) concussive rather than piercing weapons (*2*) and (ii) rapid copulation. These traits may combine infrequently in ungulates. For example, male vicuña and zebra lack hornlike weapons altogether but apparently require several minutes of frictional movements before ejaculating (*7*). Alternatives resembling blocking, while easily overlooked, may prove more common since many ungulates are sexually dimorphic in body size and weaponry.

Across taxa, at least a few other alternative strategies emphasize creative tactics. For example, alternative strategists apparently create access to females through persuasion [in chimpanzees (*8*)] and deception [female mimicry in some fishes (*9*) and feigned alarm signals in mule deer (*10*)]. A parallel to coursing occurs in some waterfowl: mated males fight other males for temporary access to their mates (*11*). The blunt bill, webbed feet, and intromittent organ characteristic of waterfowl may mean that here, as with bighorns, combat is less risky and insemination more rapid than in other taxa.

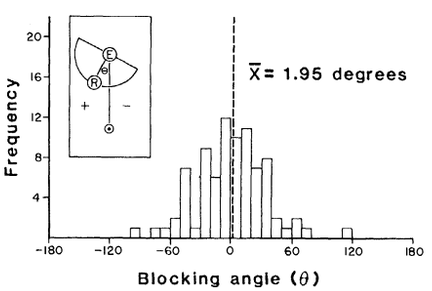

Fig. 3. Distribution of blocking angles (θ). (Inset) θ equals the angle between a line connecting the ram (R) and stationary ewe (E) and a line connecting the ewe and the center of all tending (⊙). One angle was chosen at random from each of 83 blocking episodes. The distribution is effectively normal with mean 0 and sample variance ($P = 0.78$, $Z = 0.66$, two-tailed Kolmogorov-Smirnov goodness of fit test).

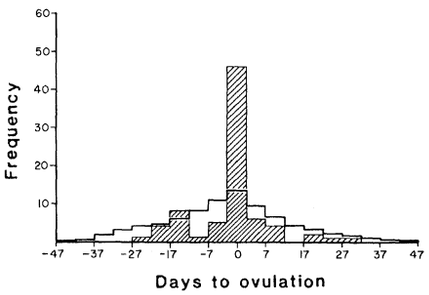

Fig. 4. Days to ovulation for first observations of blocking incidents involving single ewes ($n = 73$). Open bars give values expected if rams had selected ewes from the population at random with respect to estrus date. Ovulation was assumed to occur on the final day of estrus.

JOHN T. HOGG
Department of Zoology, University of Montana, Missoula 59812

References and Notes

1. R. I. M. Dunbar, in *Perspectives in Ethology*, P. P. G. Bateson and P. H. Klopfer, Eds. (Plenum, New York, 1982), p. 385; D. I. Rubenstein, in *Econology: The Allocation of Individual Behavior*, J. E. R. Staddon, Ed. (Academic Press, New York, 1980), p. 65.
2. V. Geist, *Mountain Sheep: A Study in Behavior and Evolution* (Univ. of Chicago Press, Chicago, 1971), pp. 208–216 and 227–228.
3. Similar interactions have been described for other caprids [G. B. Schaller, *Mountain Monarchs: Wild Sheep and Goats of the Himalaya* (Univ. of Chicago Press, Chicago, 1977), pp. 269–270; C. C. Shank, *Z. Tierpsychol.* **30**, 488 (1972)] and for giraffe [A. C. Innis, *Proc. Zool. Soc. London* **131**, 245 (1958)].
4. Dominance rank was determined by the outcome of agonistic encounters prior to rut.
5. S. A. Asdell, *Patterns of Mammalian Reproduction* (Cornell Univ. Press, Ithaca, N.Y., 1964), p. 634.
6. A. H. Harcourt, P. H. Harvey, S. G. Larson, R. V. Short, *Nature (London)* **293**, 55 (1981).
7. C. B. Koford, *Ecol. Monogr.* **27**, 153 (1957); H. Klingel, *J. Reprod. Fertil. Suppl.* **6**, 339 (1969).
8. C. E. G. Tutin, *Behav. Ecol. Sociobiol.* **6**, 29 (1979).
9. M. R. Gross and E. L. Charnov, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6937 (1980).
10. V. Geist, in *Mule and Black-tailed Deer of North America*, O. C. Wallmo, Ed. (Univ. of Nebraska Press, Lincoln, 1981), p. 210.
11. F. McKinney, S. R. Derrickson, P. Mineau, *Behaviour* **86**, 250 (1983).
12. Sample sizes for testes (grams) and body weights (kilograms), respectively, are given in parentheses: 1. *Capreolus capreolus* (2, 2) [R. V. Short and T. Mann, *J. Reprod. Fertil.* **12**, 337 (1966)]; 2. *Antidorcas marsupialis* (6, 26) [J. D. Skinner, *J. Reprod. Fertil. Suppl.* **13**, 29 (1971); V. LaChevallerie and J. H. M. Van Zyl, *Agroanimalia* **3**, 115 (1971)]; 3. *Odocoileus virginianus* (7, 7) [R. M. Robinson, J. W. Thomas, R. G. Marburger, *J. Wildl. Manage.* **29**, 53 (1965)]; 4. *Antilocapra americana* (2, 2) [G. D. Bear, in *Game Research Report, Part I* (Colorado Game, Fish, and Parks, Boulder, 1967), part 1, p. 71]; 5. *Dama dama* (8, 152): R. E. Chaplin and R. W. G. White, *J. Reprod. Fertil.* **30**, 361 (1972); D. Chapman and N. Chapman, *Fallow Deer: Their History, Distribution and Biology* (Terence Dalton, Lavenham, England, 1975), p. 32]; 6. *Damaliscus dorcas* (12, 12) [J. D. Skinner

and B. J. Huntley, *Agroanimalia* **3**, 23 (1971)]; 7. *Aepyceros melampus* (2, 2) [J. D. Skinner, *Zool. Afr.* **6**, 75 (1971)]; 8. *Oreamnos americanus* (4, 4) [R. E. Henderson and B. W. O'Gara, *J. Wildl. Manage.* **42**, 921 (1978)]; 9. *Cervus elaphus* (14, 14) [B. Mitchell, D. McCowan, I. A. Nicholson, *J. Zool.* **180**, 107 (1976)]; 10. *Ovis canadensis* (3, 3) [unpublished museum records, University of Montana, Missoula]; 11. *Tragelaphus strepsiceros* (4, 4) [J. D. Skinner and B. J. Huntley, *Zool. Afr.* **6**, 293 (1971)]; *T. angasi* (3, 3) [J. L. P. L. Tello and R. G. Van Gelder, *Bull. Am. Mus. Nat. Hist.* **155**, 323 (1975)]; 12. *Kobus defassa* (19, 10) [C. A. Spinage, *J. Reprod. Fertil.* **18**, 445 (1969); H. P. Ledger, *East Afr. Wildl. J.* **2**, 159 (1964)]; 13. *Alces alces* (10, 97) [J. M. Peek, *J. Wildl. Manage.* **26**, 360 (1962); P. Schladweiler and D. R. Stevens, *J. Mammal.* **54**, 772 (1973)]; 14. *Bison bonasus* (1, 1) [K. Swiezynski, *Acta Theriol.* **13**, 511 (1968); J. Gill, *ibid.*, p. 499]; 15. *Giraffa camelopardalis* (15, 18) [A. J. Hall-Martin, J. D. Skinner, B. J. Hopkins, *J. Reprod. Fertil.* **52**, 1 (1978); A. J. Hall-Martin, *J. Wildl. Manage.* **41**, 740 (1977)].
13. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1981), p. 231.
14. I thank J. Alcock, F. Allendorf, J. Byers, R. Hutto, D. Janzen, D. Jenni, J. McAuliffe, L. Metzgar, B. O'Gara, R. Redmond, A. Rutberg, A. Sheldon, and P. Watson for advice and criticism, and B. Hogg and P. Sykes for aid in data collection. Supported by the Montana Department of Fish, Wildlife, and Parks, the Wildlife Management Institute, the National Rifle Association, the Boone and Crocket Club, the Montana Cooperative Wildlife Research Unit, the University of Montana Zoology Department, and the U.S. Fish and Wildlife Service.

7 November 1983; accepted 21 March 1984

1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Destroys Dopamine Neurons in Explants of Rat Embryo Mesencephalon

Abstract. *Explants of embryonic rat mesencephalon were grown in organotypic culture. Addition of 10 μM 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the culture medium for 4 to 7 days resulted in loss of dopamine cell bodies and fiber outgrowths, as observed by fluorescence histochemistry. At the same time, the cultures showed decreased uptake of tritium-labeled dopamine. However, no signs of generalized toxicity were evident when the explant cultures were viewed by light and phase-contrast microscopy. These results show that MPTP exerts a relatively selective destructive action in dopamine neurons in vitro, similar to the action observed in humans and monkeys in vivo. Pargyline (10 μM), a monoamine oxidase inhibitor, protected the dopamine neurons in the explants. Organotypic cultures provide an experimental model for the study of the properties of MPTP in vitro.*

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a syndrome indistinguishable from Parkinson's disease in humans and monkeys. Several instances of parkinsonism were reported in human subjects after self-administration of illicit samples of meperidine analogs that were contaminated with MPTP (*1, 2*). Subsequent studies with monkeys (*3, 4*) showed that intravenous or intraperitoneal administration of MPTP resulted in destruction of the dopaminergic cell bodies in the substantia nigra and loss of dopaminergic innervation in the caudate-putamen. Loss of cell bodies in the substantia nigra was also reported for one human subject (*1*). Cellular destruction appears to be specific for nigrostriatal neurons; noradrenergic and serotonergic neurons are spared.

Other animal species, including the rat, cat, and guinea pig (*5*), respond to MPTP either mildly or not at all, despite injection of higher doses of the drug. Thus, the monkey has served best as a model for the study of the degenerative effects of MPTP. In an effort to provide a more accessible and less expensive experimental model, we have evaluated the response of rat substantia nigra neurons in explant organotypic cultures exposed in vitro to MPTP. We now report that exposure of the cultures to MPTP resulted in destruction of dopamine neurons and that there was no other evidence of toxicity to the cultures as a whole.

Explant cultures were established from embryonic rat midbrain (15 to 16 days of gestation) and grown on cover slips coated with collagen in Maximow chambers with double cover slip assemblies (*6*). The ventral midbrain area from a single embryo was used on each cover slip. In such cultures the dopaminergic neurons grow, extend processes, and exhibit some of the normal characteristics of dopaminergic neurons in vivo (*7*). The neurons synthesize dopamine, and homovanillic acid, a dopamine metabolite, accumulates in the medium. In addition, a high-affinity uptake and storage system for tritium-labeled dopamine is present.

On day 8 in vitro, MPTP (10 μ*M*) was incorporated into the feeding medium (*6*) and applied to the cultures. The feeding medium was changed thereafter twice weekly. Control cultures were fed with medium that did not contain MPTP. The cultures were exposed to MPTP for either 4 or 7 days and analyzed subsequently at either 24 hours or 5 days after the drug was withdrawn. Phase-contrast light microscopy of both living and fixed cultures, as well as catecholamine histofluorescence and the uptake of [^{3}H]dopamine, were used to assess the effects of the drug on the cultures.

No generalized toxic effect of MPTP was observed. A large number of axons were present in the outgrowth of cultures treated with MPTP, and their appearance did not differ from untreated cultures of the same age (Fig. 1, A and B). Cultures treated with MPTP contained fewer neurons within the explants, but the surviving neurons appeared normal (Fig. 1, C and D). To identify the dopamine neurons within the explants, we incubated cultures with α-methylnorepi-

Table 1. Uptake of [^{3}H]dopamine by cultures of ventral midbrain from rat embryos after exposure to MPTP (10 μ*M*) for 4 to 7 days. In each experiment, three to four cultures were used for each treatment protocol; with one exception, results are pooled from two experiments. Pargyline was present, where indicated, at 10 μ*M*. Uptake was measured at 37°C for 10 minutes with 10 n*M* [^{3}H]dopamine (New England Nuclear; 45.4 Ci/mmol).

Treatment	Accumulation of [^{3}H]dopamine (picomoles per culture)			
	Measured 1 day after cessation of treatment		Measured 5 days after cessation of treatment	
	Mean ± standard error of the mean (*n*)	Percent of control	Mean ± standard error of the mean (*n*)	Percent of control
	*Experiment 1**			
Control	193 ± 29 (4)	100	340 ± 88 (6)	100
MPTP (4 days)	118 ± 13 (4)	61	119 ± 16 (6)	35
	Experiment 2†			
Control	214 ± 19 (8)	100	411 ± 70 (6)	100
MPTP (7 days)	55 ± 10 (8)	26	72 ± 25 (8)	18
	Experiment 3			
Control			491 ± 104 (8)	100
Pargyline			451 ± 42 (8)	92
MPTP (7 days)			72 ± 20 (8)	15‡
Pargyline + MPTP (7 days)			283 ± 37 (8)	58§

*For comparison of experimental and control cultures, $P < 0.05$ (two-tailed Student's *t*-test). †For comparison of experimental and control cultures, $P < 0.001$ (two-tailed Student's *t*-test). ‡$P < 0.001$ (Student-Newman-Keuls test for comparison of MPTP-treated and control culture). §$P < 0.025$ (Student-Newman-Keuls test for comparison of pargyline plus MPTP to MPTP alone).