nutrient still in the stomach and thus presumably not entered into energy metabolism nonetheless can effectively control feeding so that the animal compensates accurately for the energy content of this yet to be absorbed nutrient.

Satiety cannot simply be a response to volume or caloric concentration in the preloads, since we have controlled for these features individually and found them not crucial. Presumably an integration of several mechanisms, including the capacity to monitor calorie concentration and gastric distention, is active here. Liebling *et al.* (*7*) have shown in rats with gastric fistulas that a small amount of nutrient infused directly into the duodenum will effect a transient inhibition of ongoing intake. Sharma and others (*8*) have demonstrated that gastric distention alone is sufficient to inhibit intake on a short-term basis and reported the existence of stretch receptors mediated by vagal afferent fibers. Hunt and Stubbs (*9*) have reported that in humans gastric emptying time is directly related to caloric concentration and postulated the existence of "energy receptors" within the small bowel. Thus, there is evidence for the existence of mechanisms which can monitor the nature and volume of nutrient present within the gastrointestinal tract.

These mechanisms and perhaps others in liver (*10*) and brain (*11*) must function with close integration, as our results demonstrate that satiety is the behavioral outcome of a regulatory system capable of monitoring and controlling caloric ingestion. This system can be of such accuracy as to rival that of the controls on the vital autonomic functions such as blood pressure and respiration. The existence of such an accurately graded phenomenon for the control of caloric ingestion in primates raises questions as to how such a system operates in relation to available body stores, questions that are pertinent to such conditions as hypothalamic hyperphagia and the human disorders of obesity and anorexia nervosa.

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References and Notes

1. N. E. Miller, C. J. Baily, J. A. F. Stevenson, *Science* **112**, 256 (1950).
2. J. Panksepp, *Physiol. Behav.* 7, 381 (1971); D. W. Thomas and J. Mayer, *J. Comp. Physiol. Psychol.* **66**, 642 (1968); E. R. Adair, N. E. Miller, D. A. Booth, *Commun. Behav. Biol.* **A2**, 25 (1972).
3. N. Roland, M. J. Meile, S. Nicolaidis, *Physiol. Behav.*, in press.
4. R. W. Reynolds and J. Kimm, *J. Comp. Physiol. Psychol.* **60**, 438 (1965).
5. P. R. McHugh, J. Gibbs, J. D. Falasco, T. Moran, G. P. Smith, *Brain*, in press.
6. D. A. Booth, *Physiol. Behav.* **9**, 199 (1972); C. M. Liu and T. H. Yin, *ibid.* **13**, 231 (1974).
7. D. S. Liebling, J. D. Eisner, J. Gibbs, G. P. Smith, *Fed. Proc.* **33**, 259 (1974).
8. K. N. Sharma, in *Handbook of Physiology*, C. F. Code, Ed. (American Physiological Society, Washington, D.C., 1967). sec. 6, vol. 1, p. 225; H. D. Janowitz, *Am. J. Med.* **25**, 327 (1958).
9. J. N. Hunt and D. F. Stubbs, *J. Physiol.* **245**, 209 (1975).
10. S. C. Woods *et al.*, *Psychol. Rev.* **81**, 26 (1974).
11. J. Mayer, *Ann. N.Y. Acad. Sci.* **63**, 16 (1955).
12. This work was supported by PHS grant 1 RO1 AM 18554-01 and the General Research Fund, University of Oregon Health Sciences Center.
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Sexual Inhibition Is Reduced by Rostral Midbrain Lesions in the Male Rat

Abstract. *Electrolytic lesions in a circumscribed area of the rostral midbrain of rats shortened the inhibitory period following ejaculation, thereby increasing the number of ejaculations achieved in 1-hour tests. These lesions also interrupted the dorsal norepinephrine bundle as reflected in a 63 percent reduction in telencephalic or cortical norepinephrine.*

Most contemporary theories dealing with central nervous system regulation of sexual behavior of male mammals postulate the existence of inhibitory neural processes which interact with excitatory mechanisms in producing the distinctive species-typical copulatory pattern (*1*). While the bases for such hypotheses are largely indirect and subject to alternate interpretations, one feature of the copulatory pattern that has been most widely cited as reflecting inhibitory processes is the postejaculatory interval, a prolonged period of sexual inactivity and unresponsiveness to the female which follows ejaculation. In the rat, the species studied most extensively, little information exists regarding the location or nature of the postulated inhibitory mechanisms. In one study, large midline lesions, destroying a poorly identified area at the junction of the diencephalon and mesencephalon, produced accelerated copulatory performance, including an attenuation of the postejaculatory interval (*2*). However, attempts to localize the effective site have thus far led to inconsistent results (*3*).

In the course of our investigations into the contribution of ascending catecholamine systems to the normal expression of male copulatory behavior, lesions were made to disrupt the dorsal norepinephrine (DNE) bundle before it descends into the hypothalamic medial forebrain bundle (*4*). Our results indicate that bilateral destruction of a circumscribed area (see Fig. 1) within the rostral midbrain region through which the DNE bundle projects (*4*) reliably shortens the postejaculatory interval to the extent of increasing the number of ejaculations achieved in 1-hour tests.

Sexually experienced male Long Evans rats (300 to 350 g) were given a minimum of three preoperative and four postoperative mating tests of 1-hour duration, spaced at 10-day intervals. Tests were conducted during the dark phase of a reversed 12-hour-light, 12-hour-dark cycle. Males were tested with ovariectomized, estrogen- and progesterone-treated females previously screened for sexual receptivity.

Thirty-one males received bilateral, electrolytic lesions (*5*) aimed at interrupting the DNE bundle at the rostrocaudal level of the interpeduncular nucleus. At this level the DNE bundle shows maximum separation from the other biogenic amine–containing pathways (*4*). Six males served as unoperated controls and nine underwent the sham procedure in which an electrode was lowered to a point 5 mm below the surface of the skull and then withdrawn.

The dramatic and unexpected change in some measures of copulatory activity obtained with the first five lesioned animals led us to replicate the experiment three times over a period of 24 months. Lesioned rats in each replication showed a significant mean increase in ejaculation frequency (ejaculations per test) relative to all controls ($P < .001$ in each case, by t-test). The effect was confirmed by a chi-square analysis.

Subsequently, as a means of determining whether some lesions might be more effectively placed than others, the following classification method was employed. Two rats were selected that showed substantial increases in ejaculation frequency and were used as "criterion rats" for histological grouping, preliminary to statistical analysis. Their brains were serially sectioned and stained by the Weil method. Composite, bilaterally symmetrical maps of lesion location were constructed for several rostrocaudal planes corresponding to the König and Klippel atlas (*6*) and spanning the combined longitudinal extent of lesions in both animals. These maps, which represented only the areas of destruction

common to both sides of the brain in either animal, were then used as "criterion lesions" for classifying the remaining animals. An animal was placed in group A if its lesions bilaterally touched or overlapped the criterion lesions at any rostrocaudal level; otherwise the animal was put in group B. One hundred percent agreement of lesion classification was obtained by two of us based on independent ratings made without knowledge of the behavioral data. The criterion animals, designated as group C, were excluded from all statistical analyses utilizing this classification.

The overall effect of bilateral destruction to the area indicated in Fig. 1 was to increase the number of ejaculations per test session (Table 1). Group A + C animals showed an average increase of 37 percent in ejaculation frequency over the preoperative tests. For group A animals this change was significantly different from group B at the $P = .05$ level, and different from the combined sham-lesioned (S) and unoperated (U) control animals at $P < .001$. (Sham-lesioned and unoperated animals were combined for all analyses after null tests showed no population differences.)

To better define the nature of the behavioral change which resulted in this increase in ejaculation frequency, analyses were conducted on standard measures of copulatory performance for the first three ejaculations per test session (Table 1). Significant lesioning effects were found for the mount frequency and the inter-intromission interval associated with the first ejaculation of the test session. The most striking effect, however, was a decrease in the postejaculatory interval of from 38 to 47 percent relative to prelesion values for the first three ejaculations of group A animals. In this respect, group A differed significantly from group S + U and from group B.

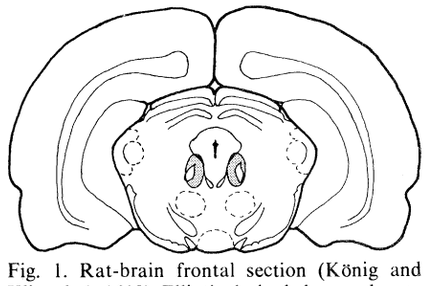

Fig. 1. Rat-brain frontal section (König and Klippel, A-1610). Elliptical, shaded areas denote the probable outer limits of tissue destruction effective for the observed increases in sexual behavior. This "effective region," with a rostrocaudal extension from A-3290 to A-1020, was estimated from the lesions of eight rats showing postoperative increases in copulation. The clear area within the effective region shows a sample pair of small lesions that caused an increase in copulatory performance.

We often observed extreme changes in the copulatory behavior of individual animals that are not reflected in the group analyses presented above. For example, one of the criterion rats went from an average of 5.8 ejaculations per hour before surgery to 11.5 after surgery. In some of the extreme tests attempts were made to determine whether the behavioral ejaculation (*7*) was accompanied by expulsion of seminal fluid. While an ejaculate was clearly identified in a few cases (for example, for behavioral ejaculations 11 and 12 in one test), it was not in other instances. Thus, "ejaculation" as used in this report means the behavioral response without necessarily implying physiological ejaculation in every case.

While ejaculation frequency and postejaculatory interval were the only behavioral measures showing a strong and consistent lesioning effect across groups, other aspects of copulation were sometimes altered in a dramatic fashion. In postoperative tests, three animals repeatedly showed ejaculations preceded by only one intromission with a number of ejaculation latencies of less than 15 seconds. One of these had no intromissions before each of several ejaculations. Typically, the normal rat shows six to ten intromissions before ejaculation, with ejaculation latencies of about 200 seconds (see Table 1).

The effect of our lesions on the DNE bundle was assessed by biochemical determination of telencephalic or cortical norepinephrine (NE) values (*8*). Group A males who were assayed had a 63 percent depletion of NE relative to sham and unoperated controls ($P < .001$). Group B animals who were assayed showed a significantly smaller NE depletion (26 percent). Group A rats assayed for caudate dopamine had no significant depletion. The placement of our lesions made it unlikely that the ventral NE bundle could have been damaged (*4*), and therefore hypothalamic NE was not measured. Although there was an equally low probability that serotonin-containing pathways could have been damaged, we nevertheless measured telencephalic serotonin in several lesioned animals, since depletion of serotonin has frequently been correlated with increased sexual behavior (*9*). There was no significant depletion of this amine.

To summarize, lesions of a circum-

Table 1. Mean values averaged over preoperative tests for 18 measures of sexual behavior, together with percent change after lesioning. Ejaculation frequencies are per 1-hour test. Mount and intromission frequencies are per ejaculation. Latencies, in seconds, are reciprocals of means of reciprocals. Mount and intromission latencies are measured from start of test to first mount and intromission, respectively. Ejaculation latency is time before each ejaculation, beginning with the first associated intromission. Inter-intromission interval is average time between intromissions preceding an ejaculation. Postejaculatory interval is measured to the first subsequent intromission. The lesions of group A and group B rats overlapped and failed to overlap, respectively, the criterion region (see text). Criterion rats (group C) were omitted from statistical analyses. Sham-lesioned (S) and unoperated rats (U) showed no population differences in behavior and were combined. Between-group *t*-tests were based on difference scores between preoperative values (averaged over three to four tests) and postoperative values (four tests).

Groups	Ejac. freq. (hr^{-1})	Mount latency (sec)	Introm. latency (sec)	Ejaculation latency (sec)			Mount frequency (per ejaculation)			Intromission frequency (per ejaculation)			Inter-intromission interval (sec)			Postejaculatory interval (sec)		
				1st*	2nd	3rd	1st*	2nd	3rd	1st*	2nd	3rd	1st*	2nd	3rd	1st*	2nd	3rd
A + C (N = 26)																		
Before	4.3	17	24	290	150	170	6.7	2.9	3.9	9.8	5.8	6.6	33	30	32	340	420	520
Change	+37%	−15%	−26%	−34%	−31%	−40%	−45%	−38%	−20%	−9%	−18%	−22%	−24%	−16%	−17%	−47%	−38%	−41%
B (N = 5)																		
Before	4.1	14	19	290	160	170	5.5	2.7	3.5	10.0	5.2	5.1	31	34	35	380	470	530
Change	+6%	+33%	+18%	−37%	−31%	−28%	−30%	+10%	−11%	−16%	+35%	−5%	−19%	−28%	−21%	−19%	−14%	−14%
S + U (N = 15)																		
Before	4.2	17	22	380	190	200	4.8	2.1	4.1	10.2	5.3	6.7	42	37	36	350	420	490
Change	−4%	+7%	+1%	−22%	−24%	−24%	+14%	+8%	−19%	−4%	−8%	−19%	−8%	−17%	−12%	−2%	−4%	+1%
A vs. S + U	†						‡						§			‡	†	†
B vs. S + U																‡		
A vs. B	§															§	§	§

*Refers to ejaculations within the session. †$P \leq .001$. ‡$P \leq .01$. §$P \leq .05$.

scribed area in the rostral midbrain reduced the inhibitory aftereffects of ejaculation (postejaculatory interval) with a resulting increase in the number of ejaculations achieved in 1-hour tests. These lesions also interrupted the DNE bundle, as reflected in a 63 percent reduction of telencephalic NE (*10*).

The apparent relationship between NE depletion and altered copulatory performance may have resulted from (i) destruction of the DNE bundle, or (ii) destruction of a system in close proximity to the DNE bundle but functionally unrelated to it. Since telencephalic NE depletion has also been obtained after hypothalamic medial forebrain bundle lesions which impair copulation (*11*), it follows that the behavioral effects obtained in either the earlier study or the present one were produced by something other than DNE bundle damage. While a choice between these alternatives cannot be made on the basis of current information, we have observed that lesions of the type reported in the earlier study also produce substantial dopamine depletion as well as damage to the descending medial forebrain bundle (*12*).

If we assume that interruption of the DNE bundle was responsible for the behavioral changes found in the present study, this suggests that either (i) this system normally exerts a direct inhibitory effect on copulation, or (ii) the DNE system has an indirect effect on behavior through its modulation of other systems with which it is in a state of dynamic equilibrium. Partial damage of the DNE bundle may have disrupted this equilibrium, setting in motion compensatory changes in potent excitatory systems more directly involved in behavior regulation. When viewed in this way, the assignment of an excitatory or inhibitory role to the DNE bundle, as applied to a behavioral end point, loses much of its meaning. Such an interaction may occur between NE and dopamine systems, and is suggested by recent evidence at both the behavioral (*13*) and biochemical levels (*14*).

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References and Notes

1. F. A. Beach, *Physiol. Rev.* **47**, 289 (19→ R. Kurtz and N. T. Adler, *J. Comp. Physiol. Psychol.* **84**, 225 (19→ B. D. Sachs and R. J. Barfield, *ibid.* **86**, 607 (1974).
→ L. Heimer and K. Larsson, *Experientia* **20**, 460 (1964).
3. ———, *Acta Neurol. Scand.* **40**, 353 (1964); R. D. Lisk, *Science* **152**, 669 (19→ *J. Exp. Zool.* **161**, 129 (1966); *J. Reprod. Fertil.* **19**, 353 (1969); G. W. Giantonio, N. L. Lund, A. A. Gerall, *J. Comp. Physiol. Psychol.* **73**, 38 (1970); J. J. Chen and D. K. Bliss, *ibid.* **87**, 841 (1974).
4. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 1 (1971).
5. With the skull level, a 0.25-mm monopolar, platinum-irridium electrode was lowered to a point 3.5 mm anterior to the interaural line, 0.8 to 1.0 mm lateral to the midline, and 7 to 7.5 mm below the surface of the skull. A 1-ma current was passed between the electrode and a rectal cathode for 20 or 30 seconds.
6. J. F. R. König and R. A. Klippel, *The Rat Brain: A Stereotaxic Atlas* (Krieger, Huntington, N.Y.; facsimile of 1967 edition).
7. The behavioral response which normally accompanies ejaculation in the rat can be easily identified as an intromission (mounting of the female and a brief series of rapid shallow and deep thrusts terminated by a quick dismount) in which the last deep thrust is intensified and prolonged and the dismount is slower.
8. Dopamine and norepinephrine were determined by a modification of the semiautomated fluorometric procedure of P. Waldmeier and L. Maître [*Anal. Biochem.* **51**, 474 (1973)]. These procedures are based on the trihydroxyindole process described by R. Laverty and K. M. Taylor [*ibid.* **22**, 269 (1968)]. The catecholamine-containing eluates from the alumina column were converted to trihydroxyindoles with the use of a Technicon AutoAnalyzer, and the fluorescence was measured with an Aminco-Bowman spectrophotofluorometer equipped with a flow cell. Assay values (μg/g) were as follows. Telencephalic NE: control (U + S), 0.32 ± 0.04, N = 4; lesion group A, 0.13 ± 0.01, N = 9; lesion group B, 0.24 ± 0.02, N = 3. Cortical NE: control, 0.27 ± 0.01, N = 5; lesion group A, 0.09 ± 0.01, N = 8. Caudate dopamine: control 8.2 ± 0.5, N = 5; lesion group A, 9.5 ± 0.7, N = 8.
9. G. L. Gessa and A. Tagliamonte, in *Serotonin—New Vistas: Biochemistry and Behavioral and Clinical Studies (Advances in Biochemical Psychopharmacology Series*, vol. 11), E. Costa, G. L. Gessa, M. Sandler, Eds. (Raven, New York, 1974), pp. 217–228.
10. R. Y. Moore, in *Frontiers in Catecholamine Research*, E. Usdin and S. Snyder, Eds. (Pergamon, New York, 1973), pp. 767–769.
→ A. R. Caggiula, S. M. Antelman, M. J. Zigmond, *Brain Res.* **59**, 273 (1973). For a comparison of the lesion locations in the present and earlier experiment in relation to the DNE bundle, see D. M. Jacobowitz and M. Palkovits, *J. Comp. Neurol.* **157**, 13 (1974); O. Lindvall and A. Björklund, *Acta Physiol. Scand. Suppl.* **412**, 1 (1974); M. Palkovits and D. M. Jacobowitz, *J. Comp. Neurol.* **157**, 29 (1974).
12. Since the preparation of this report for publication, we have learned that R. J. Barfield, C. Wilson, and P. G. McDonald [*Science* **189**, 147 (1975)] have obtained a similar effect on male rat copulatory behavior with lesions located in the ventral medial lemniscus region. Owing to the difference in lesion locations, it is not presently clear whether the two findings are based on the same anatomical system.
13. C. O. Malmnäs, *Acta Physiol. Scand. Suppl.* **395**, 1 (1973); J. Masur, S. Czeresnia, H. Skitnevsky, E. A. Carlini, *Pharmacol. Biochem. Behav.* **2**, 55 (1974); S. M. Antelman, H. Szechtman, P. Chin, A. E. Fisher, *Brain Res.*, in press.
14. H. Nybäck, *Acta Pharmacol. Toxicol.* **30**, 372 (1971); T. H. Svenson and B. Waldeck, *Eur. J. Pharmacol.* **7**, 278 (1969); I. Creese and S. D. Iversen, *Brain Res.* **83**, 419 (1975).
15. Supported by PHS grants MH 16581 and MH 24114 and by the A. W. Mellon Educational and Charitable Trust. We thank Robert Eibergen for assistance on histology and Marina Lin for biochemical analysis. Hormones were generously supplied by Dr. Preston Perlman, Schering Corporation, Bloomfield, N.J.

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Glucose-6-Phosphate Deficiency and Inhibition by NADPH: A Self-Contradictory Argument

In his discussion of hemolytic anemia resulting from genetic deficiencies of human glucose-6-phosphate dehydrogenase (G6PD) (*1*) Yoshida cited the traditional explanation: These variants "cannot generate enough NADPH in red cells to maintain an adequate concentration of reduced glutathione." The impairment in ability of G6PD-deficient cells to maintain normal levels of reduced glutathione has been recognized for many years; and NADPH (reduced nicotinamide adenine dinucleotide phosphate), the product of G6PD, is known to be necessary for keeping glutathione in the reduced form. In his review, however, Yoshida argues that these same "hemolytic variant enzymes are strongly inhibited by NADPH of physiologic concentration . . ." and "therefore, in the presence of low concentrations of NADP and relatively high concentrations of NADPH as in the red cells, the hemolytic variants such as Manchester, Tripler, and Alhambra, can scarcely function (Table 4) although their 'red cell G6PD activity' is more than 20 percent of normal." The activities of normal and variant G6PD's in the presence of high ratios of NADPH to total NADP (0.82 to 0.99), such as are known to exist in normal red cells, were shown in Yoshida's table 4.

These statements are self-contradictory. The red cell cannot have levels of NADPH which are, at the same time, both normal and deficient. We would not ordinarily call attention to so obvious a discrepancy, except for the repetition of this contradiction (*2*).

Consideration of the maximum velocities and either table 3 or figure 2 in (*1*) reveals that the competitive inhibitor constants (K_i's) of the NADPH-sensitive variants are not low enough to explain the greater clinical severity of these variants, if the ratio of NADPH to total NADP is less than half the normal ratio. Although they are competitively inhibited by NADPH much more readily than other G6PD-deficient variants, their maximum activities are much greater. Thus, a high ratio of NADPH to the total NADP leads to the contradiction mentioned, whereas a low to moderate ratio of NADPH to total NADP (*3*) leads to refutation of a major point in the article, that concerning the significance of low K_i's in certain variants of human G6PD with clinically severe symptoms.

An argument might be made that Yo-