metabolic events that result in changes in lactate, amino acids, ammonia, and water and alterations in the level of idiogenic osmoles (*8*). These changes may be harmful to oligodendrocytes, the myelin-forming cells of the central nervous system. As suggested by Bass and Sima (*9*), oligodendrocytes in the brainstem are particularly sensitive and may be preferentially involved in osmotic stress.

Another potential mechanism for demyelination may be related to vasogenic edema secondary to osmotic opening of the blood-brain barrier. Whether a result of endothelial cell shrinkage (*10*), enhanced transvesicular transport (*11*), or some other mechanism, edema is seen after osmotic stress (*10, 12*) as well as in CPM (*13*). Edema may cause demyelination (*14*). If this mechanism is correct, it might explain the localization of the lesions in our experimental animals as well as in human CPM. Demyelination would be expected to be maximal in areas where gray matter with its rich vascular supply (source of edema fluid) is interwoven with white matter (where the bulk of the myelin is contained). Such an admixture is characteristic of the human pons; in the rat, this pattern is prominent in the striatum, thalamus, and upper brainstem, where the lesions were found. Demyelinative lesions were not found in the pons in the rat, which has far more white than gray matter.

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15. Supported by the Research Service of the Veterans Administration. We thank K. P. Bell for technical assistance.
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8 September 1980; revised 4 November 1980

## Vaginocervical Stimulation Selectively Increases Metabolic Activity in the Rat Brain

Abstract. *Vaginocervical stimulation affects progesterone secretion, sperm transport, sexual receptivity, locomotion, and perception of pain in female rats. In this experiment, vaginocervical stimulation produced statistically significant increases in the metabolic uptake of carbon-14-labeled 2-deoxy-D-glucose in the following brain areas (ordered by magnitude of uptake): medial preoptic, mesencephalic reticular formation, bed nucleus of stria terminalis, dorsal raphe, and globus pallidus. The results provide information about the concurrent processing of sensory stimulation by several neural areas and indicate that the medial preoptic area is a receiving area for copulatory stimulation.*

Stimulation of the female rat's vaginal cervix by the male during coitus (or copulomimetic stimulation) has several behavioral and physiological consequences: it potentiates the lordotic posture of the female (*1*), initiates the neuroendocrine reflex necessary for subsequent pregnancy (*2*), releases luteinizing hormone (*3*), facilitates transport of sperm from the vagina into the uterus (*4*), and inhibits somatic reflexes, locomotion, and pain (*5*).

It is known that the pelvic nerves, which innervate the cervix and portions of the vagina, are the parts of the peripheral nervous system that mediate several of these effects (*6*). However, the central nervous system mediators are not as well known, even though the classical techniques of recording, lesion, and stimulation have all been applied to this problem. For example, electrophysiological recordings show altered firing rates in response to copulomimetic stimulation in brain areas extending from the nucleus reticularis gigantocellularis caudally to the medial preoptic area rostrally. These responsive areas include parts of the hypothalamus, the median eminence, the amygdala, the hippocampus, the midbrain, and the lateral septum (*7, 8*). Lesions of the reticular formation, the dorsal raphe, the lateral vestibular nucleus, the habenula, the hypothalamus, the preoptic area, the lateral septum, the corpus striatum, or the cerebral cortex affect lordosis behavior (*9*). Electrical or electrochemical stimulation of several hypothalamic areas can initiate the progestational state (*10*).

We used the $^{14}$C-labeled 2-deoxy-D-glucose (2-DG) method of autoradiogra-

Table 1. Relative concentration of $^{14}$C in selected brain areas. The data were derived from optical density measurements of 2-deoxy-D-[$^{14}$C]glucose autoradiographs. Each value is the ratio of the $^{14}$C concentration in each gray matter structure to the $^{14}$C concentration in the average white matter for that animal. For each structure, the increase of relative $^{14}$C concentration in the stimulated females is expressed as a percent of the control value.

| Structure | Relative concentration of $^{14}$C | | Percent increase over control value |
|---|---|---|---|
| | Vaginal stimulation | No stimulation | |
| Medial preoptic area | 1.89 ± 0.18 | 1.38 ± 0.21 | 37.0* |
| Reticular formation | 2.05 ± 0.26 | 1.67 ± 0.19 | 22.8† |
| Nucleus of the stria terminalis | 1.98 ± 0.16 | 1.62 ± 0.15 | 22.2† |
| Locus coeruleus | 1.89 ± 0.51 | 1.55 ± 0.29 | 21.9 |
| Lateral preoptic area | 2.17 ± 0.24 | 1.79 ± 0.29 | 21.2 |
| Anterior hypothalamus | 1.60 ± 0.08 | 1.32 ± 0.18 | 21.2 |
| Dorsal raphe | 2.24 ± 0.17 | 1.85 ± 0.21 | 21.1† |
| Habenula | 2.89 ± 0.23 | 2.40 ± 0.37 | 20.4 |

*$P < .014$ by Mann-Whitney $U$ test (one-tailed). †$P < .029$ by Mann-Whitney $U$ test (one-tailed).


0036-8075/81/0306-1070$00.50/0  

phy (*11*) to identify anatomic structures involved in the processing of copulomimetic cervical stimulation.

The subjects were virgin female rats (Charles River CD Sprague-Dawley strain; 210 to 265 g) that had had at least two consecutive 4-day estrous cycles. To equalize their hormonal status (*12*), the animals were ovariectomized and treated with exogenous hormones (*13*). Only rats that displayed an excellent lordotic posture in response to manual palpation of the flanks were included in the study. An indwelling catheter was inserted in the right jugular vein so that the isotope could be introduced without stressing the animal (*14*). Animals were provided with food and water ad libitum. However, on the morning of the experiment they were deprived of food for 6 hours in order to equalize their blood glucose concentrations.

During the experiment, each animal was awake and gently restrained with cloth wrappings. A smooth-tipped metal rod 2 mm in diameter, attached to a vibrator, was inserted into the vagina of four experimental females until the rod just touched the cervix (*15*). Four (yoked) control females heard the vibrator but did not receive any vaginocervical stimulation. For the first 5 minutes the vibrator was switched on for 15 seconds per minute. A pulse of 2-DG (15 $\mu$Ci per 100 g) was then infused through the catheter and followed by a flush of saline. For the next 10 minutes stimulation continued at a rate of 15 seconds per 30 seconds. For the final 35 minutes the cervical tapping continued for 15 seconds per minute (*16*).

After this stimulation the animal was killed by an overdose of anesthetic and the brain was prepared for x-ray autoradiography (*17*). The optical densities of the autoradiographs from 44 brain structures were measured with a microdensitometer (Gamma Scientific, aperture of 0.25 mm) and were automatically converted to $^{14}C$ concentrations by comparison to six calibrated standards.

Nuclei known to be involved in reproductive phenomena (*7–10*), as well as others, were assessed in all females. After autoradiography, brain structures were identified (*18*) by staining the sections with cresyl violet. On each section at least four densitometric readings were taken for each anatomic area; these readings were averaged over all the sections in which the structure appeared, even if there was heterogeneity in the metabolic rate for an area across sections.

To compare the concentrations of $^{14}C$ in the same brain structure in stimulated and unstimulated females, we needed to control for individual differences in the brain's overall metabolism of glucose. We therefore expressed the data as ratios in which the numerator is the $^{14}C$ concentration of each of the 44 gray matter structures and the denominator is the average concentration of $^{14}C$ in that animal's white matter (*19*). (We calculated the white matter baseline by averaging $^{14}C$ concentrations in optic chiasm, corpus callosum, anterior commissure, internal capsule, cerebellar peduncles, and hippocampal commissure.)

In 8 of the 44 brain structures metabolic activity increased by 20 percent or more in response to vaginocervical stimulation (Table 1). The medial preoptic area (MPOA) showed the greatest increase (37 percent over control values). This increase was statistically significant ($P < .014$). On the autoradiograms this area is visible as a bilateral wedge of darkening, lateral to the third ventricle and extending from the anterior commissure to the optic chiasm. It is bordered laterally by darker areas in the lateral preoptic (Fig. 1A). Autoradiographs from control females did not show this wedge-shaped area of increased activity (Fig. 1B). The stimulated females also showed statistically significant increases in activity in the mesencephalic reticular formation, nucleus of the stria terminalis, and dorsal raphe. Although the locus coeruleus, lateral preoptic area, anterior hypothalamus, and habenula showed elevated uptake of 2-DG, the increases were not statistically significant.

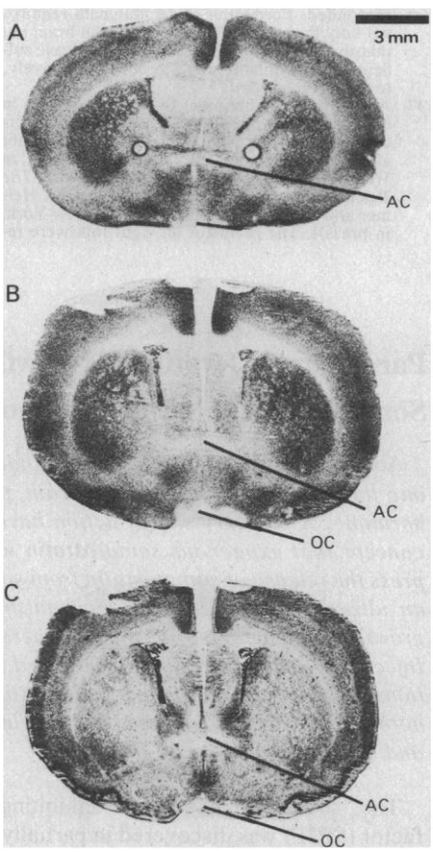


Fig. 1. (A) 2-Deoxy-D-[$^{14}C$]glucose autoradiograph from a female rat that received vaginocervical stimulation. The preoptic area is visible as the bilateral wedge of darkening extending from the anterior commissure (*AC*) to the optic chiasm (*OC*). Average relative $^{14}C$ concentration in the medial preoptic area in this animal was 2.15. (B) Autoradiograph from a female that did not receive stimulation. The medial preoptic area is pale. Average relative $^{14}C$ concentration in the medial preoptic area in this animal was 1.70. (C) Cresyl violet-stained section. This is the section from which the autoradiograph in (B) was taken. Stained sections were used to confirm the anatomic identification in the autoradiographs; note the medial preoptic area here.

For the other 36 nuclei the difference in relative $^{14}C$ concentration between stimulated and unstimulated animals was less than 18 percent (*20*). None of these differences was statistically significant, except for the 13 percent increase over control values in the globus pallidus ($P < .014$).

The brain areas that showed increased metabolism following vaginocervical stimulation have been implicated in the control of reproduction by experiments employing recording, lesion, and stimulation techniques (*7–10*). The 2-DG method of autoradiography can therefore validate the results of other experimental methods.

A major advantage of the 2-DG method over other techniques is the ability to assay concurrently a virtually unlimited number of brain areas in an individual animal without invading or destroying the integrity of the brain. This is important in discriminating among neural systems that overlap in part of their extent. The mediobasal hypothalamus–median eminence complex, for example, is a final common path for two behaviorally initiated neuroendocrine reflexes—induced ovulation and the progestational state.

Several brain structures (*7–10*) implicated in reproductive physiology by other techniques did not show increased metabolic activity in this study. This may have been due, at least in part, to the fact that 2-DG autoradiography may not detect increases in a diffusely organized nucleus, such as the nucleus reticularis gigantocellularis, in which only 4 percent of the genitally sensitive neurons respond specifically to vaginocervical stimulation (*8*).

This study demonstrates the effects of vaginocervical stimulation on metabolic

activity of multiple brain areas in individual female rats. We cannot, at present, relate a specific brain area to a specific behavioral or physiological consequence of copulatory stimulation. We speculate that the MPOA acts as a receiving area for copulatory information provided by cervical stimulation during mating (*21*). The brief stimulation from each intromission may be integrated in this brain site, even when the intromissions are widely spaced (*22*). At a later time (*23*) the MPOA is inhibited to allow the progestationally relevant nocturnal surges of prolactin to occur (*24*).

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13. For each of two consecutive weeks, estradiol cypionate (0.05 mg per 0.1 ml of oil) was injected intramuscularly two times, 48 hours apart, and was followed 24 hours later by progesterone (0.5 mg per 0.1 ml of oil).
14. One week before the experiment the rats were anesthetized with Chloropent (0.3 ml per 100 g), and one end of the catheter (Dow Silastic tubing; inner diameter, 0.020 inch; outer diameter, 0.037 inch) was inserted into the right jugular vein. The other end was capped, threaded under the skin behind the ear, and anchored to the top of the skull with dental cement. To maintain patency, catheters were filled with a 33 percent solution of polyvinylpyrrolidone in heparinized saline fortified with gentamicin.
15. The vibroengraving tool (Ideal Industries, Sycamore, Ill.) was adjusted to its slowest speed. The stimulation was not painful because (i) in preliminary work, when the vibrator was applied to the cervix of female rats, no tissue damage of the cervix or vagina was found; (ii) the animals gave no overt signs of distress during the stimulation; and (iii) vaginocervical stimulation is analgesic, not nocioceptive [E. L. Ross, B. R. Komisaruk, D. O'Donnell, *J. Comp. Physiol. Psychol.* **93**, 330 (1979)].
16. With this schedule we tried to both mimic the intermittent stimulation provided by a male rat during coitus and produce a sufficiently high level of stimulation to optimize the 2-DG method. In a preliminary study we tested 19 female rats. Six of the nine stimulated females produced deciduomata; none of the ten unstimulated animals responded. Formation of deciduomata requires not only that the stimulation reach the brain but that the anterior pituitary and ovary release sufficient prolactin and progesterone, respectively, to affect the uterus.
17. At this time the brains of two of the animals in each group were perfused in situ with 3.3 percent buffered Formalin. Such perfusion does not affect the retention of the isotope [P. J. Hand, in *Methods in Contemporary Neuroanatomy: The Tracing of Central Nervous Pathways*, L. Heimer and M. Rolands, Eds. (Plenum, New York, in press)]. The brains of all eight rats were removed and frozen in Freon (−45°C), stored at −70°C, and cut into 20 sections on a cryostat at −16° to −18°C. The sections were exposed to x-ray film (Kodak SB5) for 10 days along with a set of six methylmethacrylate standards with known concentrations of $^{14}C$.
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25. We thank H. Bradford and A. Sylvestro for their valuable technical assistance. Supported by NIMH grant I T32 MH 15092 (T.O.A.), PHS program project grant NS 10939-08 (J.H.G. and M.R.), and NIH grant HD 04522 (N.T.A.). These data were reported in preliminary form in *Soc. Neurosci. Abstr.* **4**, 339 (1978).
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28 July 1980; revised 18 November 1980

## Paradoxical Elevation of Growth Hormone by Intraventricular Somatostatin: Possible Ultrashort-Loop Feedback

Abstract. *Somatostatin, the growth hormone-inhibiting factor, when microinjected into the third ventricle of the rat brain, paradoxically induced the release of growth hormone. A pituitary site of action having been ruled out, this result supports the concept that exogenous somatostatin within the hypothalamus acts either to suppress the release of somatostatin from somatostatin-containing neurons, possibly via an ultrashort-loop feedback mechanism, or to augment release of hypothalamic growth hormone-releasing factor, thereby inducing a release of growth hormone. Injection of somatostatin into the third ventricle also decreased plasma concentrations of luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone, probably by inhibiting the release of luteinizing hormone-releasing factor and thyrotropin-releasing factor.*

The somatotropin release-inhibiting factor (SRIF) was discovered in partially purified hypothalamic extracts by Krulich *et al.* (*1*) on the basis of its ability to inhibit growth hormone (GH) secretion by the pituitary in vitro. This tetradecapeptide, isolated and characterized by Brazeau *et al.* (*2*) and renamed somatostatin, inhibits GH release both in vivo and in vitro (*2*, *3*).

Not only does SRIF inhibit GH release, but it depresses several components of the central nervous system (*4*). For instance, the microiontophoretic application of SRIF to certain hypothalamic and extrahypothalamic structures depresses neuronal firing activity (*5*). We examined the participation of centrally administered SRIF in the regulation of its own hypothalamic secretion and in that of other hypothalamic peptides. This was accomplished by monitoring the effects of central and systemic SRIF treatment on the secretion of GH, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and prolactin (PRL), all of which are pituitary hormones under the control of hypothalamic releasing or inhibiting factors. In addition, we compared in rats the responses obtained with SRIF to those obtained following injection of the decapeptide luteinizing hormone-releasing factor (LHRH).


0036-8075/81/0306-1072$00.50/0 