pBR322 and denoted pHT10, transforms cells with a 4000-fold lower efficiency (Table 1). The MSV sequences in pHT10 represent src and MuLV related sequences at the 5′ end of src. We have attributed the reduction in focus forming activity to the removal of the two 600-base pair (bp) terminal repeat sequences (TRS) bracketing the proviral genome (*13*). One example is given in Table 1. The plasmid subclone pHT13 of HT1 MSV (from the 5.3-kb *Hin*d III site to the 12.3-kb *Eco* R1 site) contains one TRS and is inactive in the transformation assay. We recombined these sequences at the *Hin*d III site to the 3.7- to 5.2-kb (*Bgl* II–*Hin*d III) MSV portion of pHT10 generating pHT22. This restored the transforming efficiency to within 10 percent of that obtained with λHT1 DNA.

When the cloned 15-kb Balb/c sarc fragment in λMsarc was tested in the transformation assay, no foci were detected (Table 1). pMS1, a sarc containing subclone of this fragment (0.7- to 4.1-kb *Bgl* II) in pBR322 produced no foci at the highest DNA concentrations tested. The Balb/c sarc sequence may fail to transform cells for several reasons. For example, a mutation in sarc could have occurred during the generation of MSV engendering it with tumorigenic activity. However, the MuLV sequences bracketing src may be responsible for activating its transforming activity (*13*). We therefore replaced the normal mouse sequences flanking the sarc region of the λMsarc insert with subgenomic portions of MSV. The stimulation of focus forming activity by the addition of MSV sequences in pHT13 to the 3′ end of the src sequence in pHT10 (pHT22, Table 1) raised the question of whether the transforming activity of Balb/c sarc could be induced in an analogous fashion. For this purpose a hybrid DNA recombinant was constructed in λgtWES and denoted λLS1; it contained λMsarc sequences from the left *Eco* RI sites to the sarc *Hin*d III site linked at the *Hin*d III site to the MSV sequences in pHT13. In contrast to the high transforming activity of pHT22, the λLS1 fragment produced only single foci at the highest DNA concentrations tested (Table 1). The most apparent difference between the inserted fragments of the inactive λLS1 and the active pHT22 resides at the 5′ end of the src sequence. The latter contains MuLV-related sequences at the 5′ end of src as does the subgenomic transforming fragment of MSV 124 (*15*). To test whether sarc would transform when attached to these MuLV-related sequences, we constructed the recombinant λLS2. The inserted fragment in this phage consists of the entire 5′ end of the MSV provirus joined at the *Bgl* I site in src to the *Bgl* I–*Hin*d III fragment from sarc (Table 1). This fragment transformed with an efficiency similar to pHT22. While we cannot exclude other reasons for the transforming activity of λLS2, these analyses suggest that MuLV sequences at the 5′ end of src are necessary for its activity (*13*).

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16. These experiments were performed under P2-EK2 physical and biological containment as required by the NIH guidelines. We thank L. W. Enquist for his gift of in vitro packaging materials and his suggestions during the course of this work. Balb/c 3T3 cells were provided by R. H. Bassin. We thank P. H. Duesberg, C. J. Sherr, and T. G. Wood for critical review of manuscript.

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Vomeronasal Pump: Significance for Male Hamster Sexual Behavior

Abstract. *Vomeronasal chemoreceptors segregated within the vomeronasal organ are important for male hamster sexual behavior. An autonomically controlled vascular pump, previously demonstrated in anesthetized animals, can transport stimuli to the receptors. Interruption of the efferent nerves controlling the pump results in behavioral deficits similar to those produced by interruption of the afferent nerves carrying information from the vomeronasal organ to the brain. Pump activation is thus a prerequisite for normal vomeronasal stimulation in behaving animals.*

Powers, Winans, and their colleagues (*1–3*) have shown that the vomeronasal organ (VNO) is involved in male hamster sexual behavior. They cut the afferent vomeronasal (VN) nerves and observed deficits in mounting and ejaculation. The effects of cutting the VN nerve were best revealed when animals were also treated with intranasal infusions of zinc sulfate to destroy or damage the main olfactory receptors (*2, 4*). Under these conditions, all animals showed deficits, whereas animals treated with $ZnSO_4$ alone showed no deficit.

We have previously investigated the physiological mechanisms by which stimulus molecules are transported to VN receptor neurons. In the hamster these neurons are sequestered inside the VNO, a tubular sac which opens at one end only through a long narrow duct onto the floor of the nasal cavity. In anesthetized animals, fluid can be moved in and out of the duct by local vasomotor

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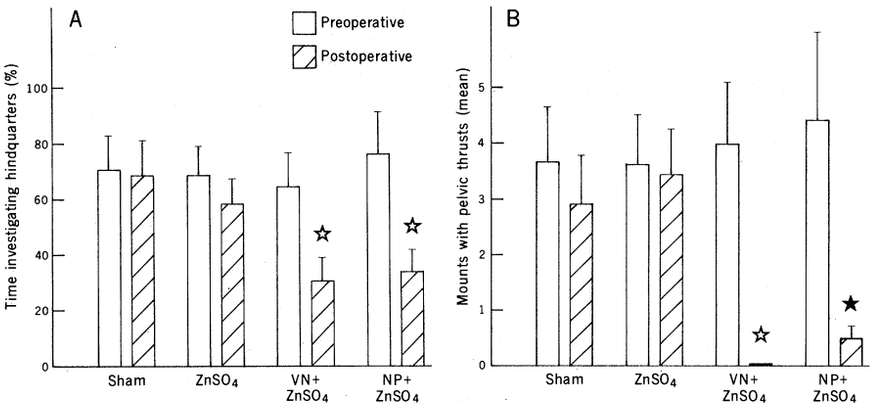

Fig. 1. Behavior of test males with surrogate females. (A) Time spent sniffing and licking the hindquarters of the animal (not forward of nor including the hind leg) expressed as a percentage of the total time spent investigating any part of the stimulus animal. Time spent mounting was not included in investigation time. (B) Attempted intromissions (mounts with appropriately directed pelvic thrusts) of test animals with surrogate females. Bars show means ± standard errors (S.E.). Postoperative changes significantly different from the change (if any) in sham animals are indicated by filled ($P < .05$) or open ($P < .01$) stars (*14*).

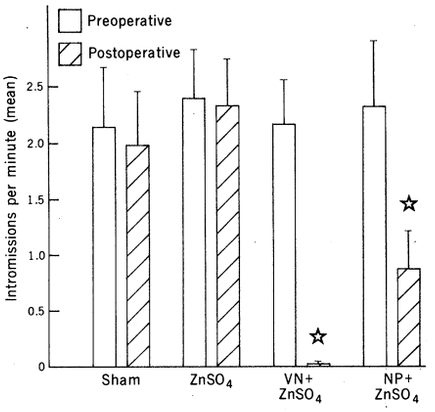

Fig. 2. Sexual behavior of test males with behaviorally receptive (cycling) females. The mean number of intromissions per minute was calculated as the number of intromissions divided by the duration of the test (*15*). Significance levels are designated as in the legend to Fig. 1 and (*14*).

movements within the VN capsule (*5, 6*). These movements are under the control of vasomotor fibers in the nasopalatine (NP) nerve. When the NP nerve was stimulated electrically, nasal fluids were sucked into the VNO. If an odor was blown continuously over the entrance to the VN duct, the activity of second order neurons in the accessory olfactory bulb (AOB) changed after NP nerve stimulation, indicating that sufficient odor molecules were sucked into the VN lumen to activate VN receptors. In the absence of NP stimulation there was no significant response to odors blown over the entrance to the duct (*5, 6*).

We have now measured sexual performance in male hamsters with severed NP nerves in order to assay the importance of the NP-activated pump for VNO stimulation in the behaving animal. If activation of the pump is necessary, the simple presence of stimulus substances in the nasal cavity is not sufficient for adequate stimulation of VN receptors. We predicted that cutting the NP nerve bilaterally would disable the pump and produce deficits in male sexual behavior similar to those produced by section of the VN nerves. We also reasoned that, since the VNO and the sensory nerves remained intact in animals with severed NP nerves there might be some residual behavior as a result of slow diffusion of stimuli through the narrow VN ducts.

Cutting the NP nerves in combination with intranasal infusion of $ZnSO_4$ resulted in deficits that are similar to (although not always as severe as) those produced by the combination of VN nerve cuts and $ZnSO_4$ treatment. The large deficits induced by the combined treatments are additional to the minimal deficits produced by $ZnSO_4$ alone.

Male hamsters [Engle Ela:ENG(SYR), 12 to 14 weeks old] were screened for normal sexual behavior and allowed to ejaculate three times with a receptive female. After preoperative data were obtained for all animals on two tests, groups of eight to ten animals were formed by matching preoperative performance scores. Three groups were given one of the following lesions each: bilateral section of the VN nerves (*7*), bilateral cautery of the NP nerves (*8*), or sham VN or NP nerve lesions (sham group). The following day, the animals with VN or NP lesions were treated with intranasal $ZnSO_4$ solution (*2, 4, 7*). At this time, a fourth group received $ZnSO_4$ as its only treatment, and the sham group received intranasal saline. Thus, there were three experimental groups in addition to the sham group.

The animals were tested 2 days after the $ZnSO_4$ treatment for sexual behavior (*9*) in two situations designed to provide different complexity in the range of cues associated with receptive females. Female hamsters in estrus produce increased vaginal discharge. This material attracts males (*10, 11*) and produces other behavioral effects including a large increase in mounting and attempted intromissions with surrogate females [such as an anesthetized male scented with vaginal discharge (*11*)]. The fully receptive female, of course, both attracts the male and incites mounting, but cues other than those associated with the vaginal discharge are probably also involved. The two tests were designed to measure induced mounting when (i) the presence of the discharge was the only specifically female cue available or (ii) when all normal female cues were available.

One test was with a scented male (*12*) and the other was with an estrous female. In the first, animals were placed with an anesthetized male that had been smeared on the anogenital area with the vaginal discharge collected from a single behaviorally receptive female (scented-male test; two trials). Animals were scored during the 5-minute test for the time spent investigating the hindquarters of the anesthetized male (as percent of total time spent investigating the stimulus animal). Mounts and mounts with pelvic thrusts appropriately directed toward the stimulus animal were also scored. We interpret the latter as attempted intromissions. In testing with an estrous female, the male was placed with a behaviorally receptive, naturally cycling female for 10 minutes or until he had achieved five intromissions (estrous-female test; two trials). The animals were scored for mounts, intromissions, and ejaculations (although few ejaculations occurred within the five-intromission limit). The lesions received by individual animals were unknown to the observers. After 7 days of testing (*9*), animals were killed and prepared for histological verification of the lesions (*13*).

The behavioral deficits recorded in the scented male test were of two types, a reduced preference for the rear of scented males (Fig. 1A) and a decrease in the number of attempted intromissions (Fig. 1B). The NP + $ZnSO_4$ group showed almost identical deficits to those seen in the VN + $ZnSO_4$ group for both measures (*14*). In the cycling female test, both VN + $ZnSO_4$ and NP + $ZnSO_4$ groups showed significant deficits in the number of intromissions and in the aver-

age number of intromissions per minute (Fig. 2) (*15*).

These data support our hypothesis. Eliminating the efferent control of the VNO pump produces deficits similar to those obtained after eliminating the afferent outflow from the VNO. There were differences, however, between the performance of the two groups in the estrous-female test. When compared with the sham group, both showed statistically significant deficits in this test, but the deficit in the VN + $ZnSO_4$ group was significantly more severe ($P < .001$) (*14*). Because the VN organ and nerves were intact in NP + $ZnSO_4$ animals, one or more of the following explanations may account for the difference between groups. (i) There may be some diffusion of small amounts of stimulus substances into the VNO during the course of the 10-minute estrous-female test. (ii) The stress of handling and encounters with other behaving animals may result in some activation of the vascular pump through fluctuations in systemic blood pressure. (iii) Changes in concentration of circulating epinephrine might also operate the pump directly (*16*), especially if the adrenergic target tissue in the VNO were subject to denervation hypersensitivity, as has been suggested for other tissues (*17*).

The deficits observed in NP + $ZnSO_4$ animals in the cycling female test are, in any case, unequivocal. In the scented male test, where many cues associated with receptive females are excluded, the results are even more striking. Here NP + $ZnSO_4$ and VN + $ZnSO_4$ animals showed almost identical deficits. Under these conditions any residual VN afferent input in the animals with NP nerve lesions seems to be insufficient to maintain the behavior. We interpret the results of these experiments as evidence that the VNO pumping mechanism previously demonstrated in anesthetized animals is necessary for normal stimulation of the VNO in behaving animals.

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7. The procedures of Powers and Winans (*2*) were used for cutting the VN nerves between the main olfactory bulbs and for administering $ZnSO_4$.
8. After being exposed through the palate, the NP nerves running along the free ventral border of the nasal septum were cauterized bilaterally.
9. Animals were tested once a day in 43 by 22 by 15 cm cages during the first 3 hours of the dark phase of a 14-hour light, 10-hour dark reversed light cycle. The two tests of sexual behavior were alternated with a test for attraction to the vaginal odor as part of a separate experiment. Animals from all four groups were divided into two sets, which received the three tests in a counterbalanced order. Tests were conducted during October.
10. The term vaginal discharge does not imply a pathological condition. It is used because the behavioral effects of the discharge may be due to chemicals that are not secreted but produced by microbial action [R. E. Johnston, *Behav. Biol.* **12**, 111 (1974); A. G. Singer, W. C. Agosta, R. J. O'Connell, C. Pfaffmann, D. V. Bowen, F. H. Field, *Science* **191**, 948 (1976); (*11*)].
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12. R. J. O'Connell, A. G. Singer, F. Macrides, C. Pfaffmann, W. C. Agosta, *Behav. Biol.* **24**, 244 (1978). Intact males anesthetized with Nembutal were smeared with freshly collected hamster vaginal discharge and placed on a mound of bedding in a simulated lordosis posture.
13. Animals were anesthetized and killed by cardiac perfusion with saline and 10 percent formalin. Heads were decalcified in 4.13 percent EDTA and embedded in paraffin. The VNO and the NP nerve regions were cut coronally at 12 to 14 μm; the bulbs were cut horizontally at 12 μm and stained with Kluver-Barrera stain. Small areas of near normal olfactory epithelium could be seen in most of our animals 9 to 10 days after $ZnSO_4$ infusion. At this time, some animals also exhibited behavioral evidence for returning olfactory function, but all of these continued to show deficits in sexual behavior. In the NP + $ZnSO_4$ group, all animals included in the analysis had no observable NP fibers remaining, but all showed intact blood vessels passing by the cauterized region to supply the VNO. In the VN + $ZnSO_4$ group, animals had complete cuts as judged by our inability to follow the remnants of the VN nerve through the region of the cut in serial 12-μm horizontal sections. We used Powers and Winans' procedure (*2*) but found that the glomeruli of the AOB had not entirely degenerated at 10- and 20-day survival times. In separate experiments with the same cutting procedure, horseradish peroxidase flushed into the nose was not transported to the AOB as it was in control animals. We therefore believe that we made complete cuts in our animals.
14. Results of all tests were analyzed by calculating the postoperative score as a percentage of the preoperative score for each animal. Each set of data was first subjected to Kruskal-Wallis analysis of variance to confirm differences between groups. Scores of different groups were then compared with Mann-Whitney U tests. Excluding those with incomplete lesions or incomplete data there were eight sham, nine $ZnSO_4$, seven VN + $ZnSO_4$, and six NP + $ZnSO_4$ animals.
15. This measure is a more accurate reflection of deficits since control animals reached five intromissions (the limit) in much less than the 10 minutes allowed.
16. Epinephrine injected into the carotid artery causes vasoconstriction in the blood vessels surrounding the VNO and results in the suction of mucus or air in through the VN duct (*6*).
17. V. B. Mountcastle Ed., *Medical Physiology* (Mosby, St. Louis, 1974), vol. 1, pp. 797–798.
18. We thank David Heath for excellent technical assistance. Supported in part by National Institute of Neurological and Communicative Diseases and Stroke fellowship NS05849 (to M.M.) and grant NS14453 (to R.J.O.).

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Differences in the Distribution of Gray and White Matter in Human Cerebral Hemispheres

Abstract. *The distribution of gray matter in the two cerebral hemispheres was determined by the xenon-133 inhalation method. There was more gray matter relative to white matter in the left hemisphere than in the right, particularly in the frontal and precentral regions. This finding suggests that the organization of the left hemisphere, relative to that of the right, emphasizes processing or transfer within regions, or both, rather than transfer across regions.*

No coherent body of data exists to explain why the left hemisphere specializes in analytic, logical, and verbal functions (*1*), whereas the right hemisphere subserves holistic, gestalt, spatial functions (*2*). Although the two hemispheres seem to contain equal amounts of neural tissue (*3*), a greater density of cells has been reported in the left than in the right hemisphere (*4*), the surface of the planum temporale is larger and the sylvian fissure is longer in the left hemisphere (*5*), and the left hemisphere is more extensively fissured than the right (*6*). These differences in themselves, however, do not give sufficient clues to the mechanisms responsible for functional differences between the hemispheres. We now present evidence, based on rates of isotopic clearance, for interhemispheric differences in amount and distribution of gray and white matter.

In a recently developed, noninvasive method for measuring regional cerebral blood flow, the subject inhales ^{133}Xe (*7*). Clearance of the xenon from the brain is measured by sodium iodide crystal detectors placed over the subject's head. We tested 36 right-handed male undergraduates who had no left-handed first-degree relatives. Eight detectors were placed over homologous regions of each hemisphere (Fig. 1). Using a two-compartmental analysis (*7*), we calculated the relative weight (w_1) of perfused gray matter, expressed as a percentage of the total weight (gray and white matter) of perfused tissue, for each location (*8*).

Values for w_1 were greater for the left than for the right hemisphere [F(1,

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