bine-treated and 2.6 ± 0.5 minutes for vehicle-treated ($P < 0.02$); intromission latency, 1.6 ± 0.3 minutes for vehicle-treated ($P < 0.02$); ejaculation latency, 6.9 ± 0.9 minutes for yohimbine-treated and 8.5 ± 0.8 minutes for vehicle-treated. The intercopulatory interval was 0.65 ± 0.04 minute for yohimbine-treated and 0.87 ± 0.08 minute for vehicle-treated. Most males that intromitted exhibited ejaculation (29 of 29 yohimbine-treated and 23 of 24 vehicle-treated). Values are the mean ± standard error of the mean.
19. E. Smith, J. Davidson, G. Gray, J. Clark, unpublished observations.
20. Mount latency, 1.5 ± 0.8 minutes; intromission latency, 2.4 ± 1.0 minutes; ejaculation latency, 8.2 ± 1.6 minutes; postejaculatory interval, 6.6 ± 0.4 minutes; mount frequency, 5.3 ± 1.2; intromission frequency, 6.5 ± 1.1; intercopulatory interval, 1.27 ± 0.17 minutes; copulatory efficiency, 0.58 ± 0.06 (n = 10). Values are means ± standard error of the mean.
21. G. Aghajanian and M. Rogawski, *Trends Pharmacol. Sci.* **4**, 315 (1983); J. Cedarbaum and G. Aghajanian, *Brain Res.* **112**, 413 (1976); S. Langer, *Biochem. Pharmacol.* **23**, 1793 (1974); H. Ueda, Y. Goshima, Y. Misu, *Life Sci.* **33**, 371 (1983); K. Starke, *Rev. Physiol. Biochem. Pharmacol.* **77**, 1 (1977).
22. I. Sanghui and S. Gershon, *Eur. J. Pharmacol.* **11**, 125 (1970); R. Papeschi, T. Sourkes, M. Youdim, *ibid.* **15**, 318 (1971).
23. L. Gyermek, *Pharmacol. Rev.* **13**, 399 (1961).
24. R. Papeschi and P. Theiss, *Eur. J. Pharmacol.* **33**, 1 (1975).
25. B. Scatton, B. Zivkovic, J. Dedek, *J. Pharmacol. Exp. Ther.* **215**, 494 (1980).
26. M. Meltzer, M. Simonovic, G. Gudelsky, *ibid.* **224**, 21 (1983).
27. A. Morales, P. Marshall, D. Surridge, J. Fenemore, *J. Urol.* **128**, 41 (1982); A. Morales, D. Surridge, P. Marshall, *N. Engl. J. Med.* **305**, 1221 (1981).
28. H. Schmitt, H. Schmitt, S. Ferand, *Arzneim.-Forsch.* **23**, 40 (1973).
29. S. Ahlenius, K. Larsson, L. Svensson, *Eur. J. Pharmacol.* **64**, 47 (1980); S. Ahlenius, J. Engel, K. Larsson, L. Svensson, *J. Neural Trans.* **54**, 165 (1982); C.-O. Malmnas, *J. Endocrinol.* **73**, 187 (1977).
30. F. Ferrari and G. Baggio, *Eur. J. Pharmacol.* **81**, 321 (1982).
31. Supported by NIH grant MH 21178. We thank S. McDonnell for assistance. Yohimbine hydrochloride was obtained commercially (Sigma).
* Present address: Department of Obstetrics and Gynecology, University of Florida College of Medicine, P.O. Box J-294 JHMHC, Gainesville 32610.

5 March 1984; accepted 21 June 1984

Enhanced Neural Response to Familiar Olfactory Cues

Abstract. *Norway rat pups have an enhanced olfactory bulb response to a familiar odor. A specific complex of glomeruli showed increased carbon-14–labeled 2-deoxy-D-glucose uptake in response to peppermint odor in 19-day-old pups exposed to peppermint on days 1 to 18 after birth, relative to control pups that had been exposed to clean air. The increased activity was not due to increased respiration of the familiar odor.*

Norway rat mothers emit an odor that attracts their young from the second through the fourth postpartum weeks (*1*), a period that corresponds to the time when the pups return to the mother to nurse (*2*). Mothers initially emit the attractant in low quantities, inducing the pups to orient toward it during the first 2 weeks (*3*). The principal source of the maternal odor is the cecotrophe portion of the maternal anal excreta (*4*). Synthesis of the cecal odor depends on cecal bacteria populations (*5, 6*), the composition and metabolic products of which differ with different diets (*7*). Since there is no single maternal odor, the pups must become attracted to the odor that they will approach through postnatal experience. Leon (*6*) found that pups raised with mothers on a particular diet are attracted specifically to the odor of mothers eating that diet. In fact, brief daily experience of young rats with either a maternal or an arbitrarily selected odor induces a preferential response by the pups (*8, 9*).

It seemed possible that this early olfactory experience could induce the developing olfactory system to have a special, perhaps enhanced, response to that odor. If one accepts the proposition that elevated use of glucose by neurons reflects neural activity and that 2-deoxy-D-glucose (2DG) uptake reflects glucose use, then 2DG autoradiography is a powerful technique for determining the locations of differentially active cells (*10*). We used this technique to determine whether there is a differential neural response to familiar and unfamiliar odors by young rats.

An artificial odor, peppermint, was chosen as the olfactory stimulus because its presentation could be controlled more precisely than maternal odors. We gave young rats experience with peppermint odor in a manner similar to that which had previously induced a strong behavioral preference (*9*). For 10 minutes each day for the first 18 days after birth, eight rat pups were exposed to peppermint-scented air delivered through a flow-dilution olfactometer (*11*). Exposure was accompanied by perineal stimulation, a procedure that mimics the licking that rat mothers do as part of their maternal care and facilitates the acquisition of an olfactory preference in neonates (*12*). Six control pups were exposed daily to clean air in the olfactometer while receiving perineal stimulation.

We then used 2DG autoradiography to determine whether these different olfactory experiences had influenced the responsiveness of the olfactory bulbs to peppermint. On day 19, both the pups experienced with peppermint and the naïve pups received, for the only time, a single subcutaneous injection of ^{14}C-labeled 2DG (200 μCi/kg). Both groups were then exposed to peppermint odor for 45 minutes, without perineal stimulation. We used a 45-minute exposure period to avoid the artifact associated with measuring unphosphorylated 2DG (*13*). At the end of the exposure period, pups were decapitated and their brains were quickly removed and frozen in Freon-12 at −40°C. Autoradiographs of the olfactory bulbs were prepared and developed according to standard techniques, which included exposing a set of calibrated ^{14}C-labeled standards with each film (*14*). The sections then were counterstained with thionin.

Autoradiographs, coded to prevent experimental bias, were analyzed with a computer-based image processor that allowed pseudocolor imaging and two-dimensional quantitative optical densitometry. Because the autoradiographs could be aligned by the image processor with the matching thionin-stained sections, 2DG uptake could be associated with specific lamina in the olfactory bulb (*15*). As a first step in quantifying 2DG uptake, the computer constructed a calibration curve that related the gray value of each ^{14}C-labeled standard exposure to its previously determined ^{14}C-labeled tissue equivalent. It then linearized this function so that the gray values of the autoradiograph could be translated into ^{14}C concentrations, and hence 2DG taken up by the tissue.

The entire bulb was scanned for areas of relatively high 2DG uptake. When an active glomerular complex was noted, sections throughout it were analyzed and the average uptake of 2DG by the complex was calculated. In every section, five readings (each of 40 pixels) were taken in each area of high activity within the glomerular layer, five readings were taken from the rest of the glomerular layer, and five more readings were taken within the periventricular core of the bulb. After the uptake of ^{14}C by an area of interest was determined, it was expressed relative to ^{14}C uptake in the periventricular core of the same section. Expressing uptake in terms of this ratio minimizes errors due to variations in section thickness or to nonuniformity of background illumination. Previous reports have shown that 2DG uptake in the core of the bulb is consistently low regardless of the odor stimulus used (*16*). Even short-term unilateral closure of a naris during odor presentation does not alter 2DG uptake in this region (*17*). We observed no significant difference in the concentration of ^{14}C in the periventricular core between experienced ($\overline{X}$ ±

standard error of the mean, 0.19 ± 0.07 μCi/g) and naïve (0.29 ± 0.02) rat pups, further justifying our use of this value as a baseline. We chose this method of semiquantitative ^{14}C-labeled 2DG autoradiography, as others have (*16, 18*), because we were interested in the relative, not the absolute, levels of glucose utilization in the olfactory bulb [(*19*), and see (*10*) for a discussion of the choice of an appropriate analysis for 2DG autoradiography]. The relative measures are, however, linearly related to glucose utilization in unanesthetized rats (*20*).

Figure 1 shows a pair of typical pseudocolor images from the same level through the olfactory bulbs of an experienced (peppermint-peppermint) and a naïve (air-peppermint) pup. In response to the peppermint test stimulus on day 19, both groups consistently showed 2DG uptake in three distinct areas in the glomerular layer located on the lateral aspect of the bulb, 1.5 to 2.1 mm from the rostral pole. Experienced pups, however, showed 64 percent more uptake throughout the three areas than did the naïve pups (Fig. 2) [$t(12) = 2.70$, $P < 0.01$]. There was no statistically significant difference between pups that were familiar (2.46 ± 0.60) or unfamiliar with the peppermint odor (2.03 ± 0.41) in 2DG uptake in the background glomerular region outside the reliably identified areas of high activity. These data demonstrate the topographical specificity of the enhanced activity and support the notion that the baseline measurements were stable.

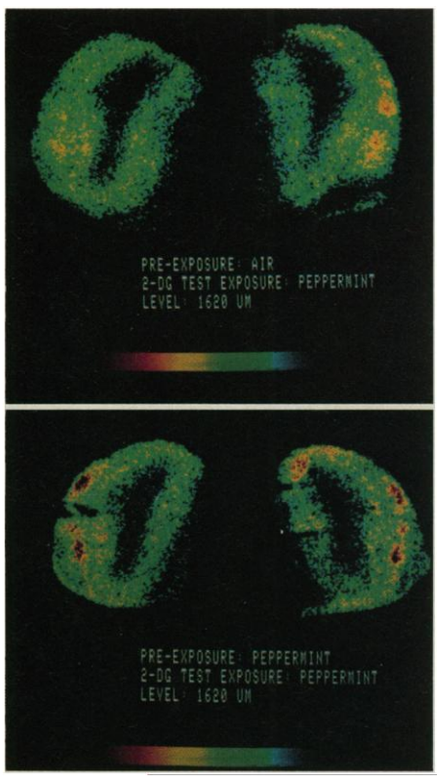

Fig. 1 (left). Computer-generated pseudocolor images of 2DG autoradiographs from coronal sections taken at the same level of the olfactory bulb of 19-day-old Norway rat pups that either had or lacked experience with peppermint odor. Red areas indicate sites of highest uptake, and blue areas indicate lowest uptake.

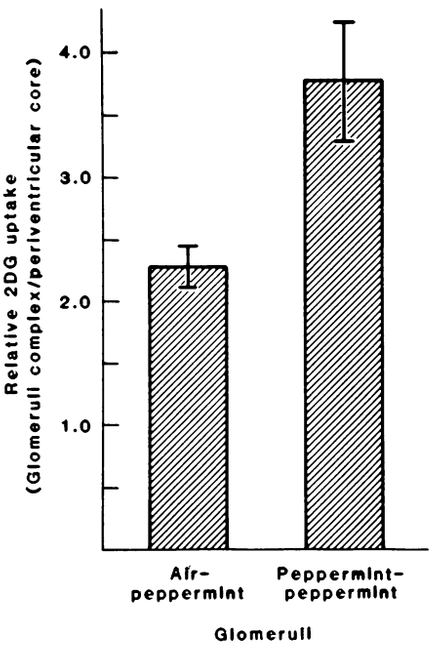

Fig. 2 (right). Uptake of ^{14}C-labeled 2DG in response to peppermint odor in a specific group of glomeruli located 1.5 to 2.2 mm from the rostral pole of olfactory bulbs from experienced and naïve rat pups. Uptake is expressed as a ratio of glomerular to periventricular core activity.

The increased neural activity suggested that the group differences in olfactory experience had induced differences in neural responsiveness. It was possible, though, that the increase in neural activity reflected greater exposure to the odor during 2DG uptake resulting from increased respiration of the familiar odor. To evaluate this hypothesis, we again exposed eight pups to peppermint odor and eight to clean air for 18 days. On day 19, we exposed all of them to peppermint for 45 minutes while we recorded their respiration rates with a pressure-sensitive transducer (*21*). There was no statistically significant difference between the respiration rates of the groups (Fig. 3). Since the control and experimental groups differed only in the odor they were exposed to during the 18 days prior to the test exposure, it seems that early olfactory experience was responsible for the enhanced neural response to the familiar odor.

Shepherd and his co-workers demonstrated that exposure to different odors leads to different patterns of 2DG uptake in the glomerular layer of both adult and young rats (*22, 23*). Increasing the concentration of the odor increases the density of 2DG uptake, suggesting that increased local activity may, in part, code for odor intensity. Perhaps the increased activity that we observed in response to familiar odors increases the intensity of the perceived odor for the pups.

The glomerular layer is diffusely organized at birth (*23, 24*); Friedman and Price (*24*) have suggested that the eventual segregation of the glomeruli may depend on neural activity. Enhanced olfactory bulb activity of the type reported here may further mold glomerular organization to reflect specific olfactory experiences. Indeed, the neural changes that occur in the development of olfactory preferences may be analogous to Freeman's (*25*) model for the formation of olfactory representations in the adult nervous system. He has suggested that the olfactory system forms templates to

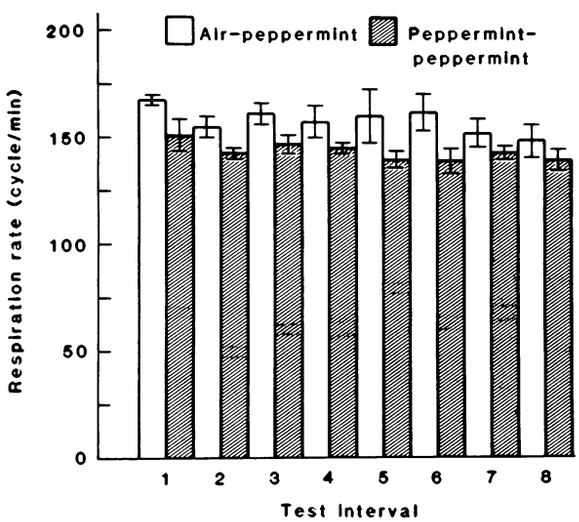

Fig. 3. Respiration rate of experienced and naïve rat pups measured during test exposure to peppermint on day 19 after birth. Measurement periods are successive 4.75-minute intervals separated by 1-minute air changes.

which incoming odors can be matched, allowing a special responsiveness to meaningful odors. The special neural responsiveness to a previously experienced odor may represent the functioning of an olfactory template.

We have shown that early olfactory experiences, of the type that reflect normal differences in sensory experience for the developing rats, can alter subsequent responses of olfactory bulbs. To date, our understanding of how mammalian sensory systems develop has been based largely on sensory deprivation or deafferentation (*26*). We hope that analyses like this one will enable us to learn more about how development is affected by normal variation in sensory experience.

ROBERT COOPERSMITH
MICHAEL LEON
Department of Psychobiology, University of California, Irvine 92717

References and Notes

1. M. Leon and H. Moltz, *Physiol. Behav.* **7**, 265 (1971); *ibid.* **8**, 683 (1972).
2. J. S. Rosenblatt and D. S. Lehrman, in *Maternal Behavior in Mammals*, H. L. Rheingold, Ed. (Wiley, New York, 1963), p. 5.
3. J. Altman *et al.*, *Dev. Psychobiol.* **4**, 97 (1971).
4. M. Leon, *Physiol. Behav.* **13**, 441, (1974).
5. W. Harder, *Verh. Dtsch. Zool. Ges.* **2**, 95 (1949).
6. M. Leon, *Physiol. Behav.* **14**, 311 (1975).
7. B. S. Draser *et al.*, *Proc. Nutr. Soc.* **32**, 49 (1973); J. A. Lampen and H. R. Peterjohn, *J. Bacteriol.* **62**, 281 (1951); J. R. Porter and L. F. Rettger, *J. Infect. Dis.* **66**, 104 (1940).
8. J. R. Alberts, *J. Comp. Physiol. Psychol.* **92**, 220 (1978); P. C. Brunjes and J. R. Alberts, *ibid.* **93**, 548 (1979).
9. M. Leon, B. G. Galef, J. Behse, *Physiol. Behav.* **18**, 387 (1977).
10. C. R. Gallistel, C. T. Piner, T. O. Allen, N. T. Adler, E. Yadin, M. Negin, *Neurosci. Biobehav. Rev.* **6**, 409 (1982); L. Sokoloff, *J. Cereb. Blood Flow Metab.* **1**, 7 (1981); P. J. Yarowski and D. H. Ingvar, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 2353 (1981).
11. We used three procedures to minimize extraneous odor experience by the pups. (i) Their mothers were maintained on a sucrose-based diet sufficient in fat (Teklad Diet TD69446, Madison, Wis.) after parturition to suppress the dominant maternal odor (*5*, *6*). (ii) Litters were kept in two vivarium rooms separate from the rest of the colony. (iii) All of the pups in a litter received the same olfactory experience but only one pup was tested from each litter to avoid odor contamination and the statistical problems associated with using littermates in the same group (H. Abbey and E. Howard, *Dev. Psychobiol.* **6**, 329 (1973)]. Pregnant (16- to 18-day) Wistar rats were obtained from Simonsen Laboratories and allowed to give birth in polypropylene cages (34 by 29 by 17 cm) lined with wood chips. Lights remained on from 0800 to 2000 hours and all animal rooms were maintained at 20° ± 1°C. Wayne Lab Blox and water were freely available until parturition, at which time dams were given unlimited test diet. Litters were culled to eight pups on day of birth. During daily exposure sessions, a flow-dilution olfactometer was used to obtain either a 1:10 dilution of saturated peppermint vapor (Schilling, Baltimore) at a flow rate of 8 liter/min or a clean airstream at the same flow rate. The airstreams were routed through separate sets of tubing and exposure containers to avoid contamination. The outlet from each stream opened into a 30-cm Tupperware container with an outlet port in its side opposite the airstream inlet. Clean wood chips lined the floor of the container and the temperature in the container was 22° ± 1°C. Each day, the litters were removed from the home cage and placed in the exposure chamber, about 10 cm from the airstream. Each pup was then individually cupped in a gloved hand so that heat loss was minimized and was held with its nose in the airstream. At the same time, the pup's perineal region was stimulated with a small sable hair brush (*12*).
12. P. E. Pedersen and E. M. Blass, *Dev. Psychobiol.* **15**, 349 (1982).
13. P. T. Kelly and T. McCulloch, *Brain Res.* **260**, 172 (1983).
14. After being removed from the skull, the brain was mounted on a cryostat pedestal with embedding matrix (Tissue-Tek, Miles) and immersed in Freon-12 at −40°C until frozen, usually 1 to 2 minutes. The frozen brain was equilibrated to the cryostat temperature of −17°C for 45 minutes. We then cut coronal 20-μm sections through the entire olfactory bulb. Each section was immediately picked up on a cover slip and placed on a slide warmer at 60°C for 5 to 10 minutes. We glued the cover slips to a sheet of cardboard and exposed them to Kodak SB-5 x-ray film for 10 days at 22°C in an exposure cassette. A set of ^{14}C-labeled methylmethacrylate standards (Amersham), previously calibrated to ^{14}C uptake in 20-μm brain sections, was exposed with each sheet of film.
15. The image processing system consisted of a PDP 11/23 computer in conjunction with a De Anza video image processor and a high-resolution video camera. The video image of the autoradiograph was digitized by assigning an eight-bit gray level value to each element of a 512 by 512 pixel array. The digitized image was then stored for further analysis. Next, a calibration function was constructed by plotting the gray levels of the exposure standards against their previously determined ^{14}C-labeled tissue equivalents. This curve was then fitted to a linear function. A new image of the brain section was created from this calibration function. Concentrations of ^{14}C could then be determined for any specified area of the section.
16. F. Jourdan, A. Duveau, L. Astic, A. Holley, *Brain. Res.* **188**, 139 (1980).
17. F. R. Sharp, J. S. Kauer, G. M. Shepherd, *ibid.* **98**, 596 (1975).
18. T. O. Allen, N. T. Adler, J. H. Greenberg, M. Reivich, *Science* **211**, 1070 (1981).
19. This method allowed us to quantify our results on a ratio scale, rather than on a rank-order index. While the latter method is appropriate for many brain studies, several factors precluded our use of it. Since the areas of interest are the darkest areas of the autoradiographs even in the control animals, a ceiling effect would prevent any differences from being observed in a rank-order index. Second, olfactory bulb 2DG autoradiographs have very few discrete structures to be ranked, unlike sections taken from other parts of the brain. Rather, one observes a relatively homogeneous band, covering the glomerular layer and extending to the granule cell lamina. Occasionally, this band is punctuated by small loci of increased uptake that corresponds to individual groups of glomeruli.
20. F. R. Sharp *et al.*, *Brain Res.* **263**, 97 (1983).
21. The apparatus used to measure respiration rate was a glass canister (29 by 11 cm) with an airtight plastic lid that was fitted with a pressure-sensitive transducer, the output of which was fed to a respiration monitor (Columbus Instruments). Odor entered the chamber as a 2 liter/min stream of a 1:10 dilution of peppermint or a clean airstream at the same flow rate, through the flow-dilution olfactometer. The airstream was admitted for 1 minute, followed by 4.75 minutes in which no air was admitted and the respiration rate was measured. This cycle was repeated for 45 minutes. Respiration rate was integrated over successive 15-second periods, and these values were averaged over each of the eight measurement intervals.
22. W. B. Stewart, J. S. Kauer, G. M. Shepherd, *J. Comp. Neurol.* **185**, 715 (1979); M. H. Teicher, W. B. Stewart, J. S. Kauer, G. M. Shepherd, *Brain Res.* **194**, 530 (1980).
23. C. A. Greer, W. B. Stewart, M. H. Teicher, G. M. Shepherd, *J. Neurosci.* **2**, 1744 (1982).
24. B. Friedman and J. L. Price, *J. Comp. Neurol.* **223**, 88 (1984).
25. W. J. Freeman, *Perspect. Biol. Med.* **24**, 561 (1981); *Biol. Psychiatry* **18**, 1107 (1983).
26. R. W. Guillery, *J. Comp. Neurol.* **144**, 117 (1972).
27. We thank T. O. Allen, N. T. Adler, M. Berns, and R. Walter for their help in initiating and completing this experiment. We thank P. Yahr for her comments on the manuscript. Supported in part by NSF grant BNS 8123107 and Research Scientist Development Award MH 0037 from NIMH to M.L. The Laser Microbeam Project facility at the University of California, Irvine, is a National Institutes of Health Biotechnology Resource Program and is supported by NIH grant RRO 1192 to M. Berns.

13 February 1984; accepted 15 May 1984

Illumination Induces Dye Incorporation in Photoreceptor Cells

Abstract. *Illumination of fly photoreceptors in the presence of the fluorescent dye Lucifer yellow initiates incorporation of the dye, which stains each cell down to its synaptic terminal. Unilluminated cells do not become stained. Experiments on animals in vivo show that selected cells can be stained without loss of viability. "Induced endocytosis" provides a plausible mechanism underlying this phenomenon.*

Several investigators have recently reported that certain classes of neurons autonomously take up various fluorescent dyes while other cells in the same matrix do not (*1*, *2*). Although this circumstance allows tagging of specific cells, the reason for its specificity remains obscure. We now report that illumination can induce photoreceptor cells to take up Lucifer yellow from the extracellular space. From a combination of optical experiments in living, unanesthetized animals and histological examinations, we infer that dye is internalized through "induced endocytosis." Beyond its use as a novel approach to the study of axonal wiring and visuotopic projection, this phenomenon may be used to enhance pharmacological treatment.

The retina of the fly's eye provides a decisive advantage in the study of a neural matrix because the array of photoreceptor inputs can be easily visualized in vivo. One method uses each corneal lenslet as a magnifying glass to observe the superimposed virtual images of the photoreceptors, an optical effect called the deep pseudopupil (DPP) (*3*). Alternatively, the corneal lenslets can be optically neutralized by a drop of water placed between the microscope objective and the cornea, which permits each individual photoreceptor cell to be seen (*4*). Using epi-illumination microscopy, one can simultaneously observe and selectively stimulate the photoreceptors.

Beneath each facet lens of the fly's eye, the unit structure called an ommatidium contains eight photoreceptor cells. Each cell has an isolated, special-