A variety of behavioral and learning handicaps have been observed in children born to mothers consuming moderate to high levels of alcohol during pregnancy (*16*), many of which have been replicated in animal models developed to study the effects and mechanisms of prenatal alcohol exposure (*17*). However, behavioral comparisons between the sexes in adult FAE animals have not been examined systematically in these studies. The data presented here indicate that such comparisons may be necessary to evaluate fully the influence of prenatal alcohol exposure in these animal models. Our results also suggest that some of the behavioral disturbances associated with prenatal alcohol exposure may result in part from an alcohol-induced disruption of perinatal androgen status.

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References and Notes

1. C. H. Phoenix, R. W. Goy, A. A. Gerall, W. C. Young, *Endocrinology* **65**, 369 (1959); H. H. Feder and R. E. Whalen, *Science* **147**, 306 (1965); R. A. Gorski, in *Frontiers in Neuroendocrinology*, L. Martini and W. F. Ganong, Eds. (Oxford Univ. Press, London, 1971), p. 237; G. Raisman and P. M. Fields, *Brain Res.* **59**, 1 (1973); J. M. Whitsett and J. G. Vandenberg, in *Early Influences*, G. Gottleib, Ed. (Academic Press, New York, 1978), vol. 4, p. 73.
2. R. W. Goy and B. S. McEwen, *Sexual Differentiation of the Brain* (MIT Press, Cambridge, Mass., 1980).
3. W. W. Beatty, *Horm. Behav.* **12**, 112 (1979).
4. J. A. Gray, S. Levine, P. L. Broadhurst, *Anim. Behav.* **13**, 33 (1965); W. W. Beatty and P. A. Beatty, *ibid.* **73**, 446 (1970); J. L. M. Dawson, Y. M. Cheung, R. T. S. Lau, *Biol. Psychol.* **3**, 213 (1975).
5. G. N. Wade and I. Zucker, *J. Comp. Physiol. Psychol.* **69**, 291 (1969).
6. D. H. Van Thiel, R. Lester, R. J. Sherins, *Gastroenterology* **76**, 1118 (1974); D. H. Van Thiel and J. S. Gavaler, *Alcoholism* **6**, 179 (1982).
7. J. J. Chen and E. R. Smith, *Horm. Behav.* **13**, 219 (1979).
8. E. S. Valenstein, J. W. Kakolewski, V. C. Cox, *Science* **156**, 942 (1967).
9. B. H. Shapiro and A. S. Goldman, *Horm. Behav.* **4**, 371 (1973).
10. J. L. M. Dawson, M. Cheung, R. T. S. Lau, *Biol. Psychol.* **3**, 213 (1975).
11. Cross-fostering has been reported to have no influence on the behavioral teratogenicity of FAE animals [G. L. Osbourne, W. F. Caul, K. Fernandez, *Pharmacol. Biochem. Behav.* **12**, 393 (1980); E. L. Abel, paper presented to the National Council on Alcoholism, Fetal Alcohol Study Group, New Orleans (1981)]. Moreover, the pair-fed controls in our study exhibited the normal sex difference in saccharin preference reported earlier (*5, 8*).
12. Saccharin consumption data were analyzed by a 2(sex) by 2(treatment) by 3(concentration) analysis of variance (ANOVA) with repeated measurements over the last factor. The analysis revealed significant main effects for sex [$F(1, 43) = 4.76$; $P < 0.05$] and concentration [$F(2, 86) = 105.57$; $P < 0.001$] as well as a significant sex by treatment interaction [$F(1, 43) = 7.84$; $P < 0.01$]. Simple effects which were tested revealed significant group by sex interactions at the 0.25 percent concentration [$F(1, 43) = 11.18$; $P < 0.002$] and the 0.50 percent concentration [$F(1, 43) = 5.27$; $P < 0.03$]. No significant effects were observed at the 1.0 percent concentration. An overall sex difference was observed for the pair-fed controls [$F(1, 43) = 12.69$; $P < 0.001$] but not for alcohol-exposed animals [$F(1, 43) = 0.19$; $P < 0.67$].
13. Litter size in the second experiment ranged from 5 to 15 animals and did not differ between ethanol-treated and pair-fed dams. Five animals from the ethanol-treated dams were stillborn compared to none from controls. Litters of more than eight animals were trimmed to eight on day 1. All animals from each litter were weighed at this time. Males and females exposed to alcohol weighed significantly less on day 1 ($P < 0.001$) than their respective pair-fed controls. Weights (mean ± S.E.M.) of each group were as follows: alcohol-exposed males ($N = 33$), 5.52 ± 0.13 g; control males ($N = 30$), 6.28 ± 0.15 g; alcohol-exposed females ($N = 28$), 5.28 ± 0.11 g; control females ($N = 23$), 5.97 ± 0.17 g. The weights of exposed animals measured on days 35 and 90 were not significantly different from controls.
14. Maze dimensions were according to K. S. Lashley [*Brain Mechanisms and Intelligence* (Univ. of Chicago Press, Chicago, 1929)]. At 100 days of age, free-feeding weight was reduced to 85 percent; animals were maintained at this level for the remainder of the experiment. Each animal was then exposed to the goal box for 2 minutes per day for four consecutive days; during these periods the animals were free to consume the food pellets which were used for reinforcement. The animals underwent three trials per day until they achieved a criterion of five errorless trials out of six. Tests were performed under low-level illumination during the second through sixth hours of the dark phase of the light cycle. Saccharin preference was tested between 90 and 100 days of age.
15. R. Kakihana, J. C. Butte, J. A. Moore, *Alcoholism* **4**, 57 (1980); A. N. Taylor, B. J. Branch, B. Cooley-Matthews, R. E. Poland, *Psychoneuroendocrinology* **7**, 49 (1982).
16. E. M. Ouellette, H. L. Rosett, N. P. Rosman, L. Weiner, *N. Engl. J. Med.* **297**, 528 (1977); A. P. Streissguth, C. S. Herman, D. W. Smith, *J. Pediatr.* **92**, 363 (1978); S. Landesman-Dwyer, A. S. Rogozin, R. E. Little, *Neurobehav. Toxicol. Teratol.* **3**, 187 (1981).
17. C. L. Randall, W. J. Taylor, D. W. Walker, *Alcoholism* **1**, 219 (1977); N. W. Bond and E. L. Di Giusto, *Psychopharmacology* **52**, 311 (1977); E. L. Abel and B. A. Dintcheff, *J. Pharm. Exp. Ther.* **207**, 916 (1978); E. P. Riley, N. R. Shapiro, E. A. Lochry, *Pharmacol. Biochem. Behav.* **11**, 513 (1979); C. D. Driscoll, J. Chen, E. P. Riley, *Neurobehav. Toxicol. Teratol.* **4**, 99 (1982).
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Vasoactive Intestinal Polypeptide-Like Substance: The Potential Transmitter for Cerebral Vasodilation

Abstract. *In vitro pharmacological studies demonstrated that exogenously applied vasoactive intestinal polypeptide (VIP) relaxes the smooth muscle cells of cat cerebral arteries, whereas substance P constricts them. Ultrastructural-immunocytochemical techniques show that a VIP-like substance is present in the large granular vesicles of nonsympathetic nerve axons and terminals in the cerebral arterial walls. These results provide strong evidence in favor of the hypothesis that a VIP-like substance is the transmitter for vasodilation in cerebral blood vessels.*

Cerebral blood vessels of several species receive vasodilator nerves (*1–5*). The nature of the transmitter for dilation, however, has not been determined. Although acetylcholine (ACh) has since its discovery been assumed to be that transmitter (*1, 6*), recent research indicates that it acts more like a transmitter for constriction in cerebral blood vessels (*3*). Vasoactive intestinal polypeptide (VIP) and substance P have been proposed as candidates for the transmitters for cerebral vasodilation. VIP-like and substance P–like immunoreactive nerves have been demonstrated in cat cerebral arteries (*7, 8*). Exogenously applied VIP and substance P induce dilation of the cat pial arteries in vitro and in vivo (*7–11*). We now report results of pharmacological and ultrastructural-immunocytochemical studies on the potential role of these peptides as transmitters for cerebral vasodilation.

The ring segments of cat cerebral arteries with or without endothelial cells were prepared (*3*). VIP relaxed cerebral arteries with or without endothelial cells (Fig. 1, A and B), and substance P rarely relaxed but frequently constricted the cerebral arteries with intact endothelial cells; substance P exclusively constricted those without endothelial cells (Fig. 1, C and D). Transmural nerve stimulation induced only vasodilation in these preparations with or without endothelial cells. In parallel studies, substance P at concentrations as low as $10^{-6}M$ consistently relaxed the rabbit ear arteries with endothelial cells ($n = 3$) and exclusively constricted those without endothelial cells ($n = 3$; Fig. 1, E and F).

These results indicate that the VIP-induced cerebral vasodilation is independent of endothelial cells. The direct effect of VIP on cerebral vascular smooth muscle is relaxation. On the other hand,

the substance P–induced vasodilation of the cat cerebral artery and the rabbit ear artery is dependent on endothelium. The direct effect of substance P on vascular smooth muscle cells of these arteries is constriction. Since the rabbit ear arteries have a thicker wall with more layers of smooth muscle cells than the cerebral arteries of the rabbit and cat (*12*), the failure of exogenous substance P to indirectly relax the cat cerebral arterial rings when endothelial cells are present is probably not due to the inaccessibility of endothelial cells to substance P. It is possible that endothelial cells of cat cerebral arteries are less sensitive to substance P than those of rabbit ear arteries. Furthermore, all nerve terminals in cat cerebral arterial walls are confined to the adventitial layer (*12*). The long distance between the nerve terminals and the endothelial cells (*3*) makes it unlikely that substance P released by nerves, like ACh (*3*), will reach the endothelial cells in sufficient concentrations to induce vasodilation indirectly. These results suggest that VIP, not substance P, is the likely transmitter.

Data from other studies, however, do not completely support the proposal that VIP is the transmitter for cerebral vasodilation. Wei *et al.* (*11*) reported that the exogenous VIP relaxed the cerebral arteries indirectly by releasing endogenous prostaglandins. However, the cerebral vasodilations induced by transmural nerve stimulation are unaffected by inhibition of prostaglandin synthesis (*2, 13*). Toda (*5*) reported that while dog cerebral arteries became tachyphylaxic to exogenous VIP, the vasodilation induced by transmural nerve stimulation in the same preparation was not affected. This indirect evidence argues against the possibility that VIP is the cerebral vasodilator transmitter.

Direct assessment of VIP's role as the transmitter for dilation is hampered, however, by the lack of a specific VIP receptor antagonist. In an attempt to find VIP in the neuronal vesicles, we have used a modified protein A–colloidal gold technique (*14, 15*). Purified protein A was labeled with colloidal gold particles

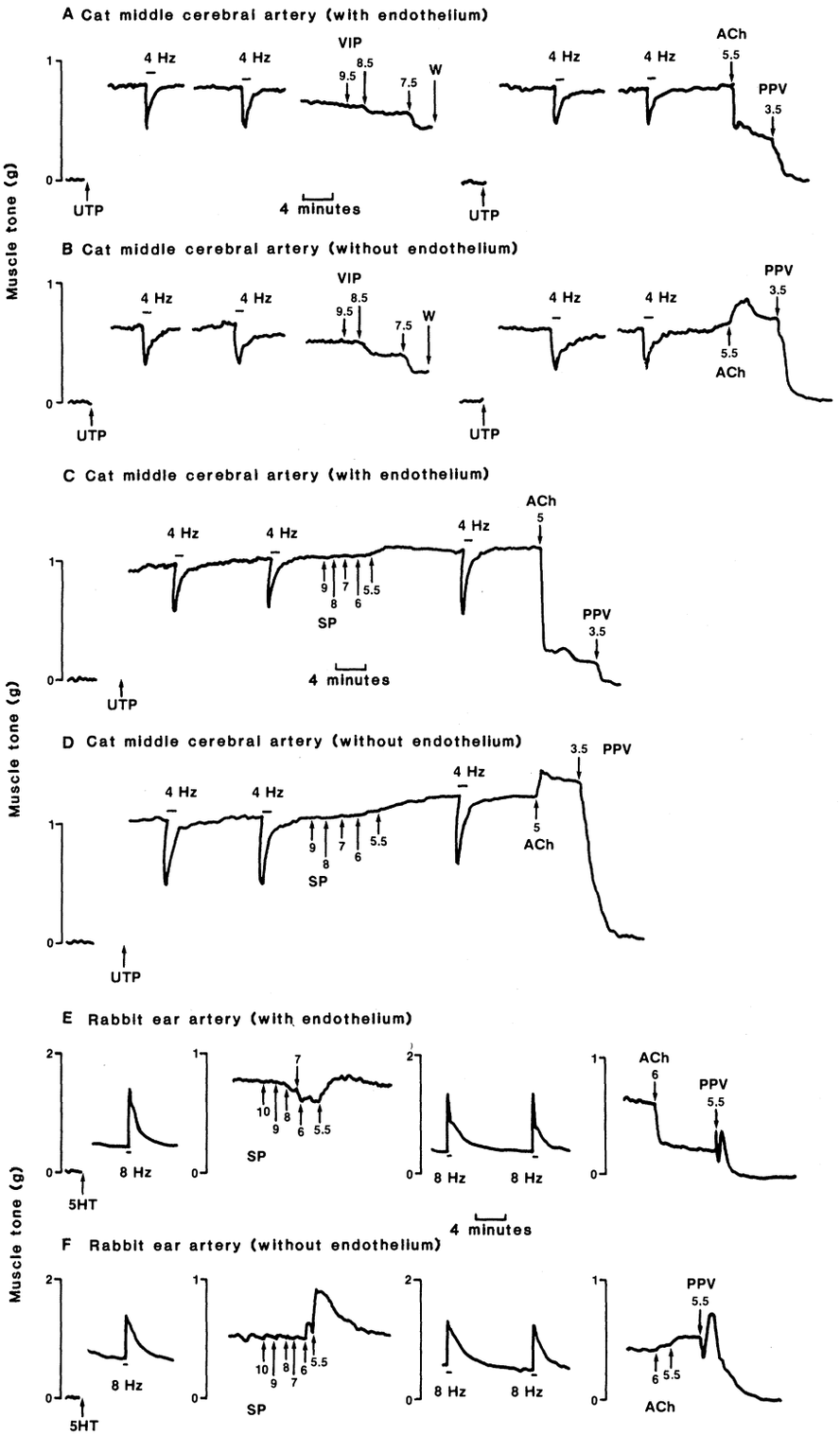

Fig. 1. Relaxation induced by transmural nerve stimulation and vasoactive intestinal polypeptide (VIP) responses induced by substance P in the cat middle cerebral and rabbit ear arteries with and without endothelial cells. Removal of the endothelial cells and measurement of tension changes of the arterial wall in vitro has been described (*3*). In the presence of active muscle tone induced by uridine-5′-trisphosphate (*UTP*), $3 \times 10^{-5}M$, VIP (Peninsula) at the concentrations applied relaxed both arteries with (A) and without (B) endothelial cells, suggesting that the direct action of VIP on the smooth muscle is relaxation. In the presence of the same active muscle tone, transmural nerve stimulation at 4 Hz consistently relaxed and substance P constricted the cat cerebral arteries with (C) and without (D) endothelial cells. In the presence of active muscle tone induced by serotonin (*5HT*) ($10^{-7}M$), the central ear artery with endothelium (E) relaxed upon application of substance P (*SP*) at low concentrations, whereas transmural nerve stimulation induced constriction exclusively. By contrast, the ear artery without endothelium (F) constricted only upon application of substance P beginning at $10^{-6}M$, whereas vasoconstriction induced by transmural nerve stimulation was not affected. In all experiments the presence and absence of endothelial cells were confirmed by giving acetylcholine (*ACh*). Acetylcholine consistently relaxed arteries with endothelial cells and constricted those without (*3*). Papaverine (*PPV*) was given to induce maximal relaxation (*3*). Abbreviation: *W*, wash. The numbers with arrows indicate negative log molar concentration of drugs in the tissue bath.

measuring 18 nm in diameter (*15*). Antisera (R-52) to synthetic VIP were raised in rabbits (*16*). The immunostaining method for ultrathin sections was similar to that described by Roth *et al.* (*14, 15*). Briefly, ultrathin sections were incubated with bovine serum albumin (3 percent) for 15 minutes then with antiserum to VIP (1:1000 dilution) for 2 hours at room temperature. The sections were rinsed with 0.01*M* phosphate buffer saline (*p*H 7.3) before incubation with gold-labeled protein A for 1 hour at room temperature. After another wash in phosphate buffer saline and distilled water, the sections were counterstained with 5 percent uranyl acetate and lead citrate.

Two sets of controls were carried out. In one, serial sections were stained with antiserum to VIP adsorbed with purified VIP; they were then incubated with the protein A–gold complex as the experimental sections were. The other control sections were incubated with the protein A–gold complex alone. Both groups were devoid of specific gold labeling (not shown).

The cerebral artery was fixed in situ by perfusing the animal, under Nembutal anesthesia (40 mg/kg intraperitoneal), with 1 percent glutaraldehyde in 0.075*M* cacodylate buffer (*p*H 7.4) for 5 minutes. The entire brain with blood vessels attached was removed. The arteries excised under a dissecting microscope were cut into small pieces and immersed in the same fixative for an additional hour at room temperature. The specimens were dehydrated in graded ethanol concentrations (50, 70, 85, 95, and 100 percent) and propyleneoxide and embedded in Polybed (Polysciences). The resin was polymerized at 60°C. Ultrathin cross sections of the arteries were cut on a microtome (Reichert) fitted with a diamond knife. The sections were mounted on slot or mesh (200) grids coated with Formvar and immunologically stained.

In the walls of all cat cerebral arteries examined (anterior cerebral, middle cerebral, and basilar arteries), VIP-like immunoreactivities were consistently found in the large granular vesicles (90 nm in diameter) in nerve terminals and axons (Fig. 2). This immunoreactivity persisted after long-term sympathetic denervation, indicating that a VIP-like substance is present in the large granular vesicles in nonadrenergic nerves. However, not all the large granular vesicles within the same nerves exhibited positive immunoreactivity. Preliminary results showed that about 30 percent of the large granular vesicles in each nerve terminal were positive to VIP immunoreactivity. This may be explained by the presence of a concentration of a VIP-like substance too low to be detected by immunocytochemistry or by the presence of some other substance in these vesicles. Similar findings have been described in guinea pig myenteric plexus (*17*).

Results of this study together with previous histochemical and biochemical demonstrations of VIP immunoreactivity (*7*) and endogenous VIP (*7, 10*) in cerebral blood vessels indicate the presence of a VIP-like substance in the neuronal vesicles. The existence of a VIP-like substance has also been suggested in neuronal vesicles in several peripheral tissues such as the submandibular salivary glands (*18*). Upon transmural nerve stimulation, a VIP-like substance can be released to induce vasodilation (*19*). Accordingly, VIP has been suggested to be the transmitter for vasodilation in the submandibular salivary glands (*19*). Thus, our findings provide evidence that a VIP-like substance is the potential transmitter for cerebral vasodilation.

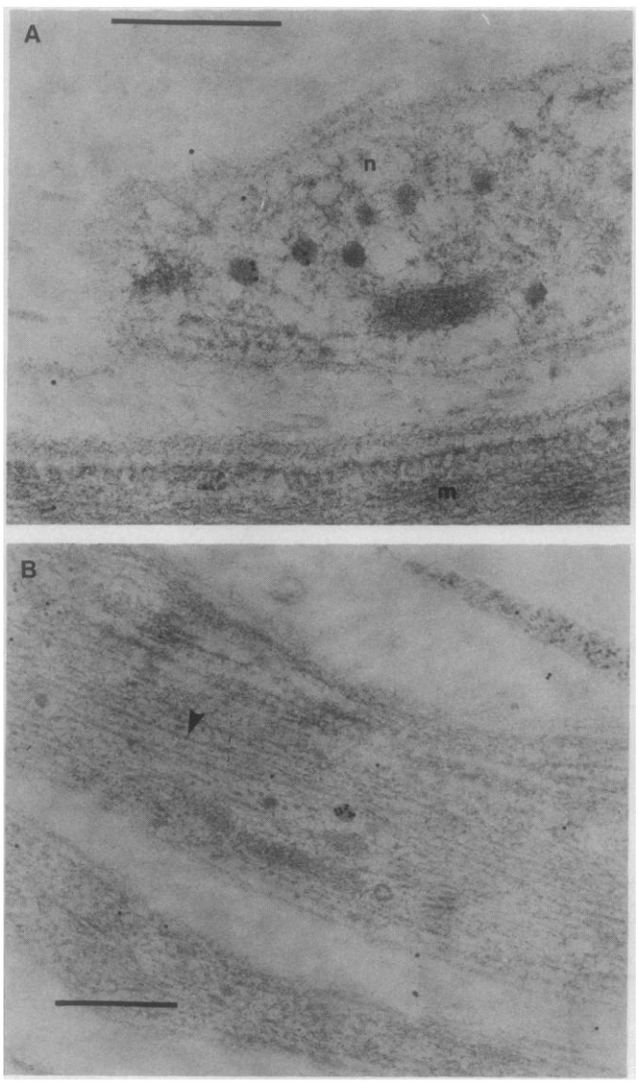

Fig. 2. Ultrathin section of the sympathetically denervated cat anterior cerebral arteries showing VIP immunoreactivity in nerve terminals (A) and axon (B). Note the specific gold labeling over the large granular vesicles. The arrowhead indicates a microtubule. Sympathetic denervation of the cat cerebral artery was performed by removing the bilateral superior cervical ganglion 1 week before the experiment. The successful denervation was confirmed by the absence of catecholamine fluorescence (*2*). Abbreviations: *m*, smooth muscle; *n*, nerve terminals. Scale bars, 0.5 μm.

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References and Notes

1. J. Chorobski and W. Penfield, *Arch. Neurol. Psychiatry* **28**, 1257 (1932).
2. T. J-F. Lee, C. Su, J. A. Bevan, *Experientia* **31**, 1424 (1975); T. J-F. Lee, W. R. Hume, C. Su, J. A. Bevan, *Circ. Res.* **42**, 535 (1978).
3. T. J-F. Lee, *Circ. Res.* **50**, 870 (1982); T. J-F. Lee, L. R. Kinkead, S. Sarwinski, *J. Cereb. Blood Flow Metab.* **2**, 439 (1982).
4. S. P. Duckles, *Circ. Res.* **44**, 482 (1979).
5. N. Toda, *Am. J. Physiol.* **243**, H145 (1982).
6. L. Edvinsson, K. C. Nielsen, C. Owman, B. L. Sporong, *Z. Zellforsch. Mikrosk. Anat.* **134**, 311 (1972).
7. L. I. Larsson *et al.*, *Brain Res.* **113**, 400 (1976); L. Edvinsson *et al.*, *Cell Tissue Res.* **208**, 135 (1980).
8. L. Edvinsson, J. McColloch, R. Uddman, *J. Physiol. (London)* **318**, 251 (1981).

9. D. D. Heistad, M. L. Marcus, S. I. Said, P. M. Gross, *Am. J. Physiol.* **239**, H73 (1980).
10. S. P. Duckels and S. I. Said, *Eur. J. Pharmacol.* **78**, 371 (1982).
11. E. P. Wei, H. A. Kontos, S. I. Said, *Am. J. Physiol.* **239**, H765 (1980); J. McCulloch and L. Edvinsson, *ibid.* **238**, H449 (1980); D. A. Wilson *et al.*, *Circ. Res.* **48**, 138 (1981).
12. T. J-F. Lee, *Circ. Res.* **49**, 971 (1981); W. R. Hume and J. G. Waterson, *Blood Vessels* **15**, 348 (1978).
13. R. J. Winquist, C. Webb, D. Bohr, *Circ. Res.* **51**, 769 (1982).
14. J. Roth, M. Bendayan, L. Orci, *J. Histochem. Cytochem.* **26**, 1074 (1978).
15. ———, *ibid.* **28**, 55 (1980); J. Roth, M. Ravazzola, M. Bendayan, L. Orci, *Endocrinology* **108**, 247 (1981).
16. C. Yanaihara *et al.*, *Biomed. Res.* **1**, 449 (1980).
17. L. Probert, J. DeMey, J. M. Polak, *Nature (London)* **294**, 470 (1981).
18. O. Johansson and J. M. Lundberg, *Neuroscience* **69**, 847 (1981).
19. J. M. Lundberg, *Acta Physiol. Scand. Suppl.* **496** (1981).
20. Supported by NIH grants HL 27763 and BRSG S07RR0543, a grant-in-aid from the American Heart Association (83-1040), funds contributed in part by the American Heart Association–Illinois Affiliate, and funds from Southern Illinois University School of Medicine. We thank E. E. Daniel for allowing us to perform the protein A–gold technique in his laboratory, S. Sarwinski for her excellent technical assistance, L. Ragel for artwork, and S. Fluckiger for preparation of the manuscript.

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Forebrain Lesions Disrupt Development But Not Maintenance of Song in Passerine Birds

Abstract. *The magnocellular nucleus of the anterior neostriatum is a forebrain nucleus of passerine birds that accumulates testosterone and makes monosynaptic connections with other telencephalic nuclei that control song production in adult birds. Lesions in the magnocellular nucleus disrupted song development in juvenile male zebra finches but did not affect maintenance of stable song patterns by adult birds. These results represent an instance in which lesions of a discrete brain region during only a restricted phase in the development of a learned behavior cause permanent impairment. Because cells of the magnocellular nucleus accumulate androgens these findings raise the possibility that this learning is mediated by hormones.*

Investigation of the neural basis of complex learned behaviors in vertebrates has been hampered by the difficulty of identifying discrete neural circuits for such behaviors: neural control of learning often seems to be broadly and diffusely represented in the brain (*1*). A salient exception to this problem is the highly localized system of hormone-sensitive central pathways that control learned vocal behavior in passerine birds (*2*). Previous investigations of this system have revealed the direct participation of discrete brain nuclei in song production by adult birds. We now report what we believe to be the first demonstration of the importance of a discrete forebrain nucleus for development of learned vocal patterns by young birds but not for production of stable song patterns by adults.

A young male zebra finch (*Poephila guttata*) learns to imitate the song of his father during a so-called "critical" or "sensitive" period of development (*3*). Song-related vocalizations are first produced at around 25 days of age; these early "subsong" vocalizations bear little resemblance to the bird's final song pattern, but vocal patterns become progressively more stereotyped between 50 and 90 days of age, and do not change thereafter. The stereotypical song patterns of adult zebra finches do not change even if birds are deprived of auditory feedback (by deafening) and of feedback from the vocal organ [by severing afferent fibers traveling from the vocal organ to the brain (*4*)]. Conversely, the song patterns of young birds are completely disrupted after deafening (*4*) and may also be affected by eliminating feedback from the vocal organ (*5*).

The system of interconnected brain nuclei that controls adult song production in passerine birds is shown in Fig. 1. Neurons in the caudal nucleus of the ventral hyperstriatum (HVc) project directly onto the robust nucleus of the archistriatum (RA). Axons of RA cells leave the telencephalon and synapse on the hypoglossal motor neurons (nXIIts) that innervate the vocal organ (syrinx). Bilateral lesions of either the HVc or the RA severely disrupt vocal behavior in adult songbirds (*2*). The magnocellular nucleus of the anterior neostriatum (MAN) projects directly onto both the HVc and the RA (*2*). Song (and possibly song learning) are androgen-dependent behaviors (*6*), and cells in the MAN, HVc, RA, and nXIIts accumulate testosterone or its metabolites (*7*).

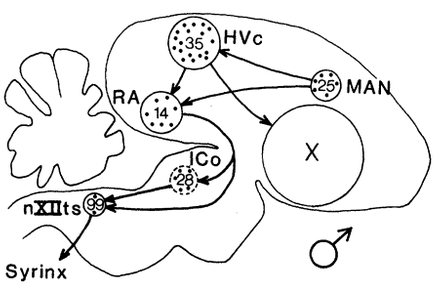

Fig. 1. Schematic drawing of the sagittal view of the neural network involved with song control in male passerine birds. Evidence for direct participation in song control has previously been reported only for telencephalic nuclei HVc and RA (*2*). Dots indicate nuclei containing androgen-accumulating cells; numbers are percentages of cells labeled within a given nucleus (*7*). Abbreviations: *HVc*, caudal nucleus of ventral hyperstriatum; *RA*, robust nucleus of archistriatum; *nXIIts*, tracheosyringeal portion of hypoglossal nucleus; MAN, magnocellular nucleus of the anterior neostriatum; *ICo*, intercollicular nucleus; *X*, area X.

The MAN has traditionally been defined as part of the song system, although nothing is known of its function. The purpose of our study was to determine the role of the MAN in the development of learned vocal behavior by juvenile male zebra finches, the production of stable song patterns by adult males, or both.

Twenty male zebra finches ranging in age from 35 to > 90 days received bilateral lesions aimed at the MAN. Birds 50 days of age and older were recorded while singing before undergoing surgery. Electrolytic lesions were produced under anesthesia (Equithesin), with monopolar stainless-steel insulated electrodes to pass anodal d-c current of 90 to 100 μA for 60 seconds. The song patterns of all birds were recorded postoperatively at approximately 2-week intervals until the birds were at least 90 days old, at which time they were killed with an overdose of anesthetic. Their brains were fixed, embedded, sectioned at 40 μm, and stained with thionin. The use of a microprojector to examine the sections allowed the exact location of the lesion to be verified. Song recordings were analyzed with a sound spectrograph (Kay Elemetrics, model 7800).

Birds with complete bilateral lesions in the MAN made when they were between 35 and 50 days old ($n = 6$) produced severely abnormal vocalizations until they were killed (Fig. 2). Their "songs" usually consisted of one or two highly abnormal notes, often produced at very low amplitude. Their notes lacked the frequency modulations characteristic of normal zebra finch song and were produced in extremely long bouts of singing which lacked normal phrasing. These abnormal song patterns appeared when birds were first recorded after surgery (typically 48 to 72 hours). In contrast, birds with lesions that missed all or most of the MAN ($n = 5$) showed normal song development (Fig. 2) (*8*). Their final song patterns consisted of short stereotyped phrases including approximately five