neurites is not 74 percent, but 89 percent, of 35 neurites. Thus directional specificity of regenerating axons of giant interneurons may result partly from secondary retraction of an initially random short neurite outgrowth with continued growth of correctly oriented sprouts.

An attractive hypothesis that remains consistent with our observations is that directional selectivity of growth is imposed by an attraction between the regenerating axons and their original target neurons. A chemoaffinity between optic nerve and optic tectum has long been suggested to explain specificity in regeneration of fish and amphibian optic nerve (*2*). Thus far there is no direct evidence for such a mechanism. In the case of the giant interneurons and dorsal cells of lamprey, such a mechanism need not act over long distances, since the axons of these neurons normally make incidental synaptic contacts with other neurons along their length.

Our previous studies on the giant reticulospinal axons suggested that their regenerating neurites tended to grow in the direction of their normal projection (*4, 6*). The present findings suggest that the tendency for regenerating neurites of giant interneurons and dorsal cells to grow rostralward reflects a specific preference for their normal pattern of projection that cannot be accounted for by nonspecific effects, such as a tendency for neurites to continue to grow in an established direction or a trophic action of the scar. We conclude that, since the spinal axons of large larval lampreys maintain directional specificity in their growth, the limited distance of axonal regeneration seen in the transected spinal cord is probably not due to a maturational loss of target-seeking ability in these axons.

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Prenatal Alcohol Exposure Alters Adult Expression of Sexually Dimorphic Behavior in the Rat

Abstract. *Saccharin preference and performance in a Lashley III maze were found to be altered in adult male and female rats that had been exposed to alcohol during gestation. Specifically, the sexual dimorphism normally observed in both behaviors was absent in fetal alcohol-exposed animals. The lack of sexual dimorphism appeared to result from a masculinization of the exposed females and a feminization of the exposed males.*

Perinatal androgen status is critical to the neurobehavioral differentiation of the male brain (*1*). Interference with the metabolism or utilization of androgens during this period produces both demasculinization and feminization of reproductive behavior patterns (*2*). In addition, an influence of perinatal androgen status on nonreproductive behavior has been established (*3*). Central nervous system organizational influences of androgens appear to be principally responsible for the expression of several nonreproductive behavioral sex differences in the adult rat, including maze learning performance, active avoidance acquisition, and saccharin preference (*4, 5*).

Alcohol is known to suppress testicular hormone production (*6*). Although exposure of fetal rats to alcohol has been reported to have no influence on their subsequent reproductive behavior (*7*), the possibility that it might influence the expression of nonreproductive, sexually dimorphic behaviors has not been examined. We now report that the normal sex differences observed in saccharin preference and maze learning are absent in fetal alcohol-exposed (FAE) animals.

Saccharin preference is a pronounced sexually dimorphic behavior that is dependent on androgen titers during the perinatal period for its adult expression. Normal adult female rats exhibit a marked preference for this nonnutritive substance when compared with males (*8*). Early postnatal administration of testosterone propionate to female animals masculinizes their adult saccharin preference, whereas estradiol benzoate administration to male animals in this period has no effect (*5*). Conversely, feminine saccharin preference is observed in male rat pseudohermaphrodites of the Stanley-Gumbreck strain, which are androgen-insensitive because of a genetic defect that causes a lack of androgen receptor (*9*).

Maze learning is also a strongly sexually dimorphic behavior, wherein male performance is statistically better than female (*3*). As with saccharin preference, the adult expression of this behavior is influenced by perinatal androgen status (*3, 10*). Therefore, if prenatal exposure to alcohol disrupts an androgen-mediated pathway during a critical period for expression of these behaviors, we postulated that adult FAE males would exhibit feminized behavior patterns relative to control males.

In the first experiment, pregnant Sprague-Dawley dams from Charles River breeders were pair-fed a liquid diet containing 35 percent ethanol-derived calories ($N = 7$) or an isocaloric liquid diet containing no ethanol (Bio-Serv) ($N = 6$). The diets were administered on day 7 of pregnancy and were continued until parturition; at this point, all dams were given free access to Purina Lab

Table 1. Results of adult behavioral tests of animals exposed to alcohol during the third week of gestation.

	Pair-fed		Alcohol-exposed	
	Male	Female	Male	Female
	*Saccharin preference**			
$\bar{x}$	11.63	25.43	16.38	20.49
S.E.M.	1.65	3.36	2.04	2.52
N	12	11	12	12
	Maze learning†			
$\bar{x}$	33.50	50.00	45.54	35.00
S.E.M.	3.19	5.49	4.31	1.56
N	14	12	13	12

*Milliliters of 0.25 percent saccharin solution consumed per 100 g of body weight. †Number of trials until achievement of criterion.

Chow and tap water. Daily maternal ethanol consumption of the experimental dams averaged 13.9 g per kilogram of body weight [± 0.68 standard error of the mean (S.E.M.)]. To avoid potential stress effects associated with handling, pups were not cross-fostered but were left undisturbed with the natural mother until weaning (*11*). No statistical differences were observed in litter sizes (range, 5 to 14 animals per litter) between ethanol-treated and pair-fed dams, nor were any gross physical deformities observed in offspring of either group. Three stillborn animals were born to one ethanol-treated dam compared to none from the control dams. Postnatal mortality rates were similar for both groups, with deaths of three offspring of alcohol-treated dams and two offspring of control dams occurring without specific cause before postnatal day 30. Offspring were weaned on day 21 and subsequently group-housed according to sex and treatment for the duration of the experiment. All animals were maintained on a 12-hour light:12-hour dark cycle with tap water and Purina Lab Chow freely available. Although there was a trend toward decreased weights in FAE animals of both sexes measured on postnatal day 31 and in adulthood, the differences were not significant at either time point.

Forty-eight animals between 90 and 130 days of age and grouped according to sex and treatment (N = 12 per group) were tested for saccharin preference. At least one animal of each sex from the seven litters of the ethanol-treated dams and from the six litters of pair-fed control dams was used in testing for saccharin preference. Twenty-four animals (N = 6 per group) were tested at a time. During testing, the animals were individually housed in the vivarium room in which they were raised under the same lighting conditions and with free access to dry Lab Chow. For the first 4 days of testing, the animals were presented with two 250-ml water bottles containing tap water whose positions were alternated daily. On days 5 through 10 of testing, the animals were presented with a choice of tap water or saccharin solution. Three saccharin solutions (0.25, 0.50, and 1.0 percent) were presented over six consecutive days to each animal in ascending order of concentration. Animals were exposed to each saccharin concentration for 2 days, and the positions of the bottles containing tap water and saccharin solution were alternated daily. Results were analyzed on the basis of the mean amount consumed over the 2-day exposure for each concentration. The amount of saccharin consumed was corrected each day to account for body weight and was expressed as milliliters of saccharin solution consumed per 100 g of body weight.

As shown in Fig. 1, female pair-fed controls exhibited a preference for saccharin across all three concentrations tested compared to their male counterparts (*12*). This pattern was absent in FAE animals. At the most preferred concentration (0.25 percent), no statistically significant sex difference was evident in the FAE animals, whereas at the 0.5 percent concentration FAE males consumed a greater amount of saccharin than FAE females. At both of these concentrations, FAE males consumed greater amounts of saccharin than pair-fed males, whereas females consumed less saccharin than pair-fed females. A trend toward normal sexual dimorphism for saccharin preference in FAE animals was evident only at the least preferred concentration (1.0 percent), but this difference did not approach statistical significance.

In a second experiment, saccharin preference and maze behavior were studied in a group of animals exposed to alcohol during the third week of gestation. Exposure to alcohol during this period approximates more closely the critical prenatal period of neural sexual differentiation in the rat. Pregnant Sprague-Dawley dams from Charles River breeders were fed the experimental or control diets described above from day 14 of gestation until parturition. Experimental and housing conditions were the same as those described above.

Offspring from six alcohol-treated and four pair-fed dams (*13*) were tested between 90 and 150 days of age for their preference for a 0.25 percent saccharin solution and for their ability to learn to negotiate a Lashley III maze (*14*). As shown in Table 1, the normal sex differences which are apparent in the pair-fed controls are not evident in the FAE animals. Pair-fed females consumed more saccharin ($P < 0.01$; analysis of variance, Bonferroni t-test) and required more trials to learn the maze than the pair-fed males ($P < 0.05$; Bonferroni t-test). No significant differences were detected between FAE males and females.

These data show that exposure to alcohol during gestation can influence the adult expression of nonreproductive, sexually dimorphic behaviors. The absence of normal sexual dimorphism in the FAE animals appears to be due to a long-term influence of prenatal alcohol exposure on both males and females. Since saccharin preference and maze learning have been shown to be influenced by perinatal androgen status (*4, 5*), the feminized behavioral patterns observed in FAE males is consistent with data demonstrating that prenatal alcohol exposure can induce alterations in testosterone metabolism or utilization (*6, 15*). Since alcohol has a direct suppressive action on testicular hormone production (*6*), the feminized behavioral patterns observed in FAE males may be mediated by direct action of alcohol on the developing fetal testes. On the other hand, potential mechanisms mediating the masculinized behavioral pattern of adult FAE females are not obvious. However, increased adrenal weights and elevated brain and plasma levels of corticosterone have been reported in 1-day-old pups after prenatal alcohol exposure (*15*), suggesting a possible role for adrenal steroids in the masculinization of these behaviors in FAE females.

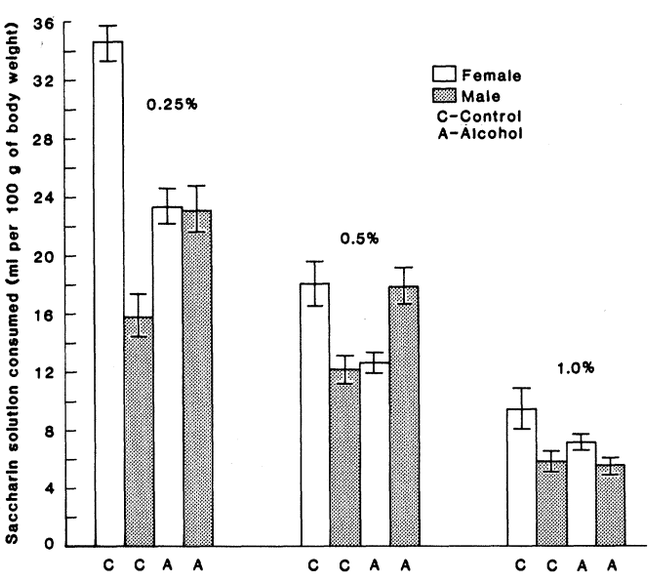

Fig. 1. The mean consumption of saccharin per 100 g of body weight at 0.25, 0.5, and 1.0 percent concentrations of saccharin. Values at each concentration represent the mean consumption over the 2 days of exposure to each concentration for animals (N = 12) of each sex in control and alcohol-exposed groups. Bars represent S.E.M.

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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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.
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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,