15. Multiple solutions are connected to the two different sides of theta tubing pulled to a 200-μm opening. The tip is shaped so that solutions from the two sides of the theta tubing when individually switched on will cover a common space. Rapid solution change is made by switching between the solutions on the two sides of the theta tubing. Although solutions were gravity-fed (20 cm), initial acceleration was provided by the small pumping action of the switch solenoids.
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The EGF Receptor Kinase Substrate p35 in the Floor Plate of the Embryonic Rat CNS

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P35 is a calcium- and phospholipid-binding protein that was originally isolated as a substrate for the epidermal growth factor (EGF) receptor tyrosine kinase and later was found to be related to lipocortin I. Immunohistochemistry was used to localize p35 to a raphe of primitive glial ependymal cells in the median one-third of the floor plate in the central nervous system (CNS) of rat embryos. The p35 appears by embryonic day 12 before the arrival of pioneering ventral commissural axons. The unexpected, discrete distribution of this protein during development opens the question of its role in neural morphogenesis.

THE CHARACTERIZATION OF SEVERAL viral oncogene products and the receptors for EGF and other growth factors as enzymes that phosphorylate tyrosine (tyrosine kinases) has led to interest in identifying substrates involved in mediating the effects of these kinases. The molecular and chemical characteristics of putative tyrosine kinase substrates have been reported (*1*); however, most studies have used cells labeled with ^{32}P in culture.

Our laboratories have focused on a 35-kD protein (p35), originally isolated from human carcinoma cells and shown to be a Ca^{2+}-dependent substrate for the EGF receptor kinase (*2*). We have used immunohistochemical methods to determine the cellular distribution of p35 in tissue sections from embryonic rats and have detected a restricted pattern of distribution in the CNS that suggests a role for p35 in neural morphogenesis.

Embryos from pregnant rats under nembutal anesthesia were fixed with Carnoy's fluid (chloroform:ethanol:acetic acid; 3:6:1), embedded in paraffin, and sectioned. Immunohistochemistry was performed with serum from rabbits immunized with human p35. This antiserum was immunoreactive with both p35 and recombinant lipocortin I (*3*); no reactivity was detected against lipocortin II. The immunostaining was blocked by prior incubation of the diluted serum with the original antigen or with p35 isolated from human placenta or with recombinant lipocortin I. Normal rabbit serum controls were negative at a 1:250 dilution of serum.

An antiserum (408) at 1:1000 dilution intensely stained a narrow sector in the ventral midline of the neural tube of a rat fetus at day 16 of gestation (E16) (Fig. 1a). The stained sector is present throughout the spinal cord and hindbrain. It has a consistent width of 40 to 80 μm and forms a longitudinal seam that occupies the median one-third of the floor plate. The ontogeny of p35 in the raphe shows a rostral to caudal progression, with stain appearing in the rhombencephalon at E11 and reaching the lumbar cord by the end of E12. The raphe staining is maximal from E15 to E18 and subsequently decreases until it disappears by postnatal day 5. P35 is not detected in any other regions of the CNS in either embryos or adults.

P35 is primarily concentrated in the bodies and processes of tall cells, each of which spans the thickness of the neural tube from the central canal to the pial surface (Fig. 1b). Both the apical and basal processes of the raphe cells form junctional "membranes" at the free surfaces. Cells in this configuration, which retain the simple pseudostratified epithelial characteristics of the primordial neural plate and early neural tube, have been called "archetypic glial ependymal cells" by comparative anatomists as far back as the early 1900s (*4*). Although the archetypic configuration is typical for all glia in lower vertebrates, it persists in adult birds and mammals only in the ventral midline of the hindbrain and spinal cord.

The absence of mitoses among the p35-positive cells is striking in comparison with the numerous mitotic figures apparent in their sister ependymal cells around the contiguous perimeter of the central canal (Fig. 1a). Studies with [^{3}H]thymidine labeling have shown that median cells of the floor plate in the rat cervical cord are born (cease DNA replication) by day E13 (*5*). Thus, the raphe cells are among the firstborn cells in the nervous system, exiting the mitotic cycle the same day as the firstborn dorsal root ganglion cells and less than a day after the first motor neurons. We do not know whether p35 expression precedes or accompanies the cessation of mitosis.

The regional differences and complexity of the raphe are best appreciated in either sagittal section (Fig. 1c) or stereo pairs of three-dimensional reconstructions from serial sections (Fig. 1d). The raphe runs caudally to the tip of the spinal cord; in the hindbrain, it undulates through both the cervical and pontine flexures to terminate rostrally at the caudal border of the mesencephalon.

The dorsal-ventral distance from central canal to pia in the CNS shows characteristic regional differences; the height of the raphe ranges from less than 150 μm in the cord to 500 μm in the brainstem (Fig. 1c). Although cellular elongation is hardly unusual in neural tissues, the height of the raphe cells at E15 is remarkable when compared to the dimensions of the same cells in adults. The spinal cord and brainstem enlarge considerably as they mature; but the distance from central canal to pia remains constant in the ventral midline, resulting in the deep ventral median fissure. The p35-positive cells attain their adult stature by day E15 and may be the first cells of the organism to do so. At a time when most cells of the embryo are prolifer-

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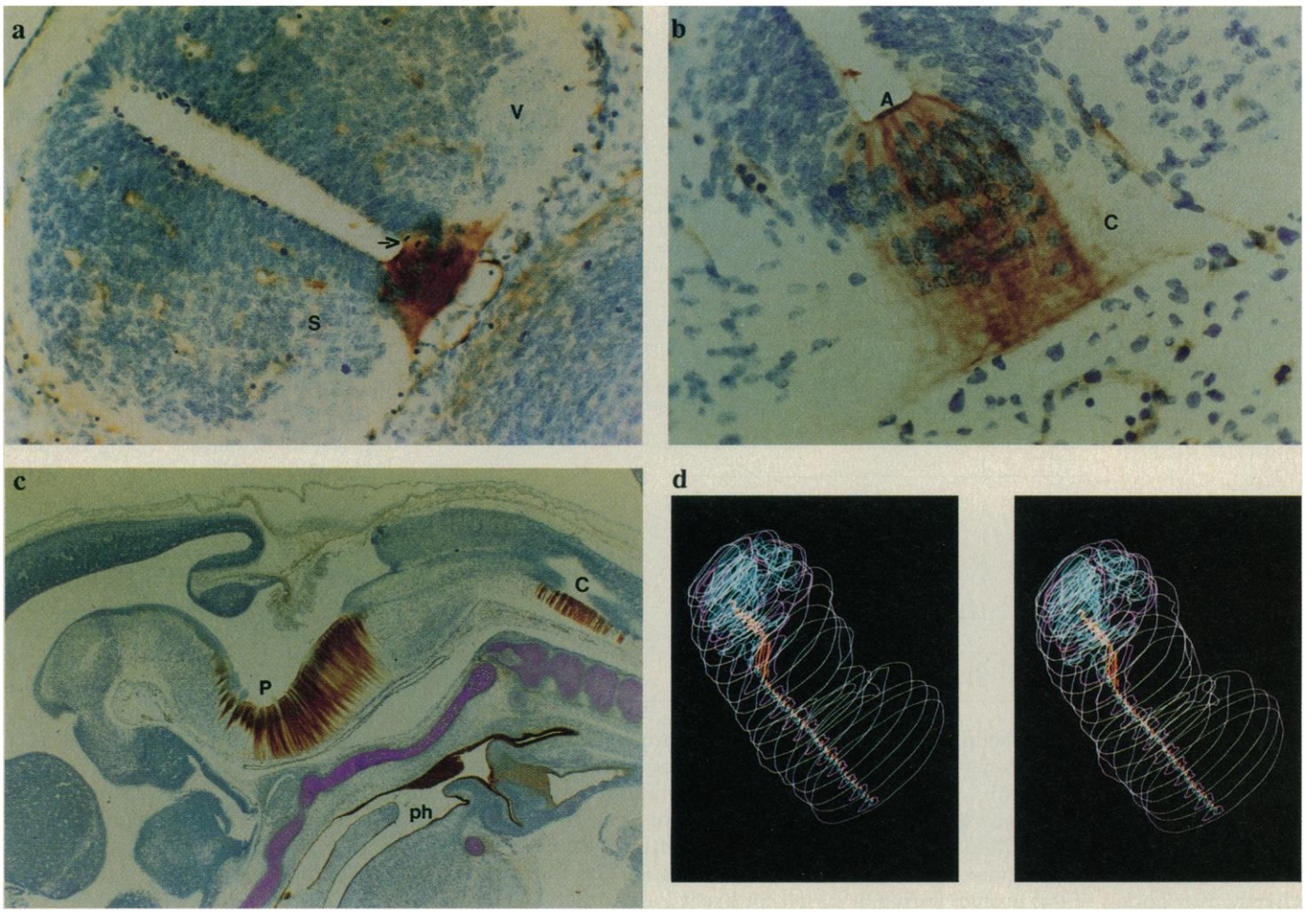

Fig. 1. (**a**) Transverse section of lumbar spinal cord from an E16 embryo illustrating that P35 is restricted to a narrow segment of the ventral midline. Sections from rat embryos fixed with Carnoy's fluid were stained to produce a brown peroxidase reaction product at p35-enriched sites (Vectastain ABC kit, Vector Laboratories, Burlingame, California), and counterstained with toluidine blue to localize nuclei. Many mitotic figures are observed around the central canal including the paramedian cells of the floor plate (arrow) that express S100 (*9*). Mitotic figures are rare among the p35-positive raphe cells. Fibers coursing toward the immature ventral commissure create stria (S) in the intermediate mantle layer. In the ventral horn (V) a few motor neurons are differentiating (×200). (**b**) Higher magnification of the ventral rhombencephalon at E14 shows that the raphe cells extend from a dense line of apical junctions (A) at the lumen of the ventricle to a less dense basal line at the pial surface. Nuclei oriented radially within the stained region are raphe cells, while the circumferentially oriented nuclei near the commissure are unidentified. Brown reaction product is primarily within cells of the raphe, but whisps of stain extend laterally along commissural processes (C). Sections fixed and stained as in (a) (×325). (**c**) Sagittal section of the anterior one-third of an E16 embryo shows segments of the raphe at the pontine flexure (P) and cervical spinal cord (C). The raphe cells are approximately 500 μm tall in the brainstem and less than 100 μm tall in the distal spinal cord. These cells retain their embryonic stature into adulthood, causing the ventral median fissure. Pharyngeal epithelium (ph) also stains for p35 at this stage. Sections fixed and stained as in (a) (×20). (**d**) Stereo image reconstructed from serial sections of the brain of E16 rat embryo with the BQ-E&S three-dimensional workstation and graphics display (BIOQUANT, R&M Biometrics, Nashville, Tennessee, and Evans & Sutherland, Salt Lake City). The raphe (orange) is present in the entire spinal cord and continues rostrally through both the cervical and pontine flexures. It stops at the fovea isthmii, a depression in the ventricular floor just caudal to the cephalic flexure. The fovea isthmii, which marks the junction of mesencephalon and rhombencephalon, is the rostral border of the floor plate in classical neuroembryology. The profiles of the ventricles and central canal (blue) are included in all sections, but the pial surface (violet) and embryo outline (grey) are shown for every third section.

ating, it seems paradoxial to have a putative mediator of the mitogenic signal expressed and enriched in the very cells that stop dividing and undergo terminal differentiation.

The location and timing of p35 expression relative to the development of neuronal pathways in the CNS are of interest with regard to the possible roles of p35 in the raphe. Major spinal sensory fiber tracts form from axons that decussate at the ventral midline commissure. In the cervical cord, the first decussating axons arise from neurons born on E12 and grow toward the commisure by E13. P35 appears in the raphe of the cervical spinal cord approximately 24 hours before the arrival of the first commissural axons.

Surface glycoprotein antigens of rat commisural fibers change as the axon growth cone passes through the midline (*6*). At high magnification (Fig. 1b), traces of stain appear lateral to the raphe, suggesting that some p35 may be carried along the surface of the decussating fibers. It is not yet known if p35 plays a role in altering the expression of antigens on commisural axons or if it plays some other role in the midline raphe.

P35 belongs to a family of structurally related proteins (lipocortins, calpactins, chromobindins, annexins, and calelectrins) that bind to phospholipids and actin in the presence of Ca^{2+} (*7*). The properties of these proteins have been reviewed (*8*), but their functions remain unknown. The restricted localization demonstrated for p35 in the raphe of the developing rat CNS may provide a logical focus for examining the function of this protein.

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Binocular Unmasking: An Analog to Binaural Unmasking?

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A visual analog to binaural unmasking was explored. The observer's task was to detect, under stereoscopic viewing conditions, an apertured sinusoidal grating added to a square patch of visual noise. In the experimental condition, the square patch of noise was presented within a frame such that the right-eye noise was a shifted version of the left-eye noise. Because of the disparity in the noise images, subjects perceived, under stereoscopic viewing conditions, that the noise patch was located behind the frame. When sinusoidal signals were added to this noise patch, the signals were clearly more detectable when the signal disparity was zero than when the signal disparity equaled that of the noise patch, demonstrating the existence of visual unmasking. Hence, under appropriate circumstances, binocular processing, in addition to providing information about depth, can also enhance the detectability of visual patterns.

THERE HAVE BEEN NUMEROUS DEMonstrations of the ability of the auditory system to use the information available in an interaural comparison to unmask a sound that is undetectable monaurally (*1*). This ability helps us, for instance, to isolate and attend to one sound source in a noisy room to the exclusion of others (the so-called "cocktail party effect"). Its importance in auditory processing is undisputed, and a number of models have been advanced to explain how the signal is extracted from the noise background (*2*). There are, however, no published reports of its direct counterpart in vision (*3*). This fact struck us as curious, especially because there is sufficient information available in interocular comparisons to permit visual unmasking in situations that are analogous to those in which auditory unmasking occurs. To show why this is the case, we (i) describe an auditory situation that produces a strong unmasking effect; (ii) show how a simple linear model of interaural processing can unmask the signal; (iii) describe an analogous binocular situation; and (iv) show how the comparable linear model of interocular processing can unmask the visual signal. Finally, we present data that demonstrate the existence of binocular unmasking in this situation.

Consider an experiment in which a band-limited Gaussian noise of unit spectral density, $n(t)$, is presented to the left ear, and a time-delayed version of the same noise, $n(t-\delta)$, is presented to the right ear, where δ is the amount of delay in seconds. The listener's task is to detect a pure tone added in phase to the noise in both ears. When the tone is presented, the left ear receives $n(t) + A\sin(2\pi ft)$ while the right ear receives $n(t-\delta) + A\sin(2\pi ft)$, where f is the tone's frequency and A is its amplitude. If we now compare detection thresholds for the case when $\delta = 0$ to the case in which $\delta = 1/(2f)$, we find that the tonal threshold when $\delta = 1/(2f)$ can be from 10 to 14 decibels (dB) lower than the tonal threshold when $\delta = 0$ (*4*). Because the binaural threshold when $\delta = 0$ is approximately equivalent to the monaural threshold for the same level of masking noise, the decibel difference in threshold is the amount of unmasking attributable to binaural processing and is often referred to as the masking level difference (MLD).

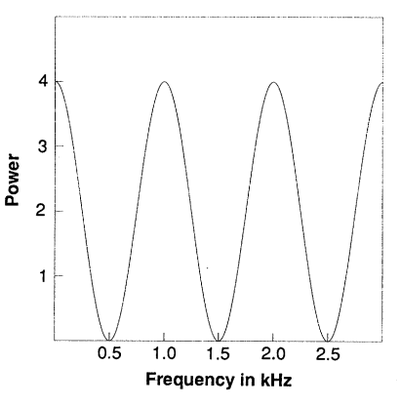

Fig. 1. Spectral density function for $n(t) + n(t-\delta)$, where $n(t)$ is a band-limited (0 to 3 kHz) Gaussian noise, and δ, the delay parameter, equals 1 ms.

A simple linear model in which the left and right ear inputs are added together illustrates how the auditory system could be extracting the signal from the noise. When noise alone is presented to the two ears, addition produces $n(t) + n(t-\delta)$. The power spectral density function G for $n(t) + n(t-\delta)$ is

$$G[f] = 2 + 2\cos(2\pi f\delta) \quad (1)$$

The plot for this function for $\delta = 1$ ms is in Fig. 1. Power density is a periodic function of frequency with peaks occurring at 0, 1000, 2000, and 3000 Hz, and troughs occurring at 500, 1500, and 2500 Hz. Therefore, if we present an in-phase pure tone to both ears with a frequency in the center of a notch (for example, 500 Hz in Fig. 1), that tone should be more easily detected than one whose frequency is at a peak (*5*).

In the visual counterpart to this auditory paradigm a square patch of two-dimensional band-limited Gaussian noise, $n(x,y)$, is presented to the left eye where n specifies the luminance value at any point (x,y) in the patch. The same noise patch is presented in the corresponding position to the right eye but shifted to the right by an amount equal to d in centimeters so that the right eye field becomes $n(x-d,y)$. When these two images, both surrounded by a square frame, are presented in a stereoscope they will fuse so that the viewer sees one Gaussian field, located behind a single square window. If the left (unshifted) and right (shifted) noise patterns are added together by the visual system, point by point, the resulting luminance pattern is given by $n(x,y) + n(x-d,y)$. The two-dimensional spectral density function for this luminance pattern varies sinusoidally with spatial frequency along its horizontal axis, that is,

$$G[\xi,\eta] = 2 + 2\cos(2\pi\xi d) \quad (2)$$

where ξ and η are the spatial-frequency

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