Subcortical projections from ectopic neocortical neurons

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ABSTRACT There is a high degree of specificity in the different connections of the neocortex. In the rodent neocortex, the characteristic band of corticospinal neurons within layer V is present at birth even though changes still occur in the areal distribution of these neurons. Disruption of neocortical development with ionizing radiation before, during, or after the production of neurons destined for layer V results in abnormally located corticospinal neurons. One abnormal location in which corticospinal neurons are found is in ectopic cell clusters beneath the cortical white matter bordering the dorsomedial aspect of the lateral ventricle. Corticospinal neurons only occur in these periventricular ectopias in adult rats irradiated on or before embryonic day 17. A second abnormal location of corticospinal neurons is between layer V and the pial surface. These scattered supragranular corticospinal neurons occur in all adult animals irradiated on embryonic days 16, 17, 18, or 19. The fact that neurons having an unusual position project to a subcortical target appropriate for one neocortical sublayer indicates that neither migratory path nor final position is essential to specifying a subcortical target. In addition, the fact that labeled corticospinal neurons are located in periventricular ectopias only when irradiation occurs on or before embryonic day 17 suggests that the initial projections of corticospinal neurons are determined early in their individual ontogeny prior to migration.

The neocortex is a complex and highly organized structure. In the adult animal, both the laminar and areal positions of a pyramidal neuron are predictive of where that neuron will send an axonal process. For example, a pyramidal neuron located in the supragranular layers will have an axon that remains within the cerebral hemispheres, while pyramidal neurons in the infragranular layers characteristically project to subcortical structures. In a similar manner, with respect to areal position, a neuron located in layer V of motor cortex will most likely project to the spinal cord, while one in the same layer of the visual cortex will most likely project to the superior colliculus or pons. This relationship between perikaryal position and axonal target is particularly striking in the neocortex. Furthermore, cortical layers can be characterized by the time of origin of their neurons. Thus, the connectivity and development of the neocortex are consistent with the hypothesis that the time and place of origin of a neuron are factors that determine a neuron's target. We decided to investigate the relationship between a neuron's cortical position and its target in the corticospinal system because there is a clear and large separation between the cell body of these projection neurons and their terminations in the spinal cord. Corticospinal neurons form a distinct band within layer V in the rat (2, 3). This laminar pattern of corticospinal neurons is present at birth, while their areal distribution continues to change postnatally (4-6). This change in the areal distribution of corticospinal neurons is associated with a reduction in the number of axonal processes in the spinal cord (7) but not with cell death (8). The early establishment of laminar location and the subsequent development of the areal distribution of corticospinal neurons indicates that laminar position may play a role in determining the subcortical projection of a neuron. To test this hypothesis, cortical development was disrupted by exposure to ionizing radiation during cortical neurogenesis to see if neurons in an abnormal position can project to their appropriate target as has been suggested by studies of the neocortex of the reeler mutant mouse (9, 10).

METHODS AND MATERIALS

Time of insemination of female Sprague-Dawley rats was determined to within 4 hr; the following morning was considered to be day 1 of gestation. Pregnant rats ranging from 16 to 19 days into gestation were exposed to a cesium-137 γ-ray source. Each animal was allowed to climb into a Mason jar that was covered with a perforated lid. The jar was placed in the exposure chamber for 28 sec (a nominal dose of 200 rads) (1 rad = 0.01 gray). The dose (as measured by thermoluminescence dosimetry with lithium fluoride rods implanted in the body cavity of adult females) was 175 ± 8 rads. The sequence of damage, reorganization, and subsequent development of the embryonic neocortex after x-irradiation has been described (11-14). After irradiation, the females delivered normally and the offspring were weaned at 21 days of age. At 30-60 days of age each offspring or control animal received an injection of several microliters of 50% horseradish peroxidase (Sigma type VI) in 5% Nonidet P-40 (15) into the cervical level of the spinal cord under anesthesia (a combination of sodium pentobarbital at 10 mg/kg, ketamine hydrochloride at 10 mg/kg, and xylazine at 3 mg/kg). Animals were perfused 72 hr later under ether anesthesia according to the method of Rosene and Mesulam (16). The brain was removed, sunk in 30% sucrose, and frozen sectioned at 40 μm. The tissue was processed with benzidine dihydrochloride according to the method of Mesulam (17). Sections were counterstained with toluidine blue and photographed under bright-field illumination. Only animals with a substantial population of labeled layer V neurons were considered suitable for comparison. In total, 36 animals were compared: 5 irradiated on E16, 16 irradiated on E17, 8 irradiated on E18, 4 irradiated on E19, and 3 control animals (E16 = embryonic day 16, etc).

RESULTS

The most striking malformations in the neocortex of prenatally irradiated adult rats are the groups of ectopic cells that border the dorsomedial aspect of the lateral ventricle (Fig. 1). There is no apparent laminar organization to these periventricular cell groups and their size progressively diminishes the later in gestation exposure to irradiation occurs. In addition to the periventricular ectopias, there are several other marked changes within the neocortex. First, the width of the cortex is reduced. This overall reduction is maximal after irradiation on E17 and appears to affect the supra- and
Fig. 1. The pattern of labeled corticospinal neurons in the normal adult (A) and adult irradiated neocortex (B, E19; C, E18; D, E17; E, E16). Arrowheads indicate the ventricular border of the ectopic cell groups. (Bright-field photomicrographs of 40-μm sections, allowed to react with benzidine dihydrochloride and counterstained with toluidine blue; bar = 0.5 mm.)
FIG. 2. Higher magnification of cortical organization illustrated in Fig. 1 (A, normal; B, E19; C, E18; D, E17; E, E16). Labeled corticospinal neurons are aberrantly located below the white matter as well as in the upper cortical layers (arrows). (Bright-field photomicrographs of 40-μm sections, allowed to react with benzidine dihydrochloride and counterstained with toluidine blue; bar = 0.5 mm.) (Inset of A) Close-up of...
infragranular layers equally. After later exposures (E18 and E19) the reduction is proportionally greater in the supragranular layers. Second, the corpus callosum is absent or substantially reduced in animals exposed on E17 and E18 but it is only slightly reduced in animals irradiated on E16 and E19. Third, lamina organization is disrupted in the interomodal portion of the neocortex, and this disruption is more pronounced in animals irradiated on E17 and E18 than in animals irradiated on E16 or E19. Thus, the disruption of cortical organization by prenatal irradiation appears to be most severe on E17 and E18. When HRP is injected into the spinal cord of a normal rat, retrogradely labeled neurons form a distinctive band in layer Vb of the dorsomedial portion of somatosensory-motor neocortex (Figs. 1A and 2A). The pattern of labeled neurons in the irradiated rats differs from the normal pattern in two ways: First, labeled neurons are found in the periventricular ectopias as well as in their normal position in layer Vb in adult rats that were irradiated on E16 and E17. The labeled neurons appear evenly dispersed throughout the ectopias in animals irradiated on E16 (Fig. 2E) and evenly distributed in layer Vb. In contrast, in animals irradiated on the following day (E17), the labeled neurons are clustered in the medial aspect of the ectopias (Fig. 2D) and labeled neurons in layer Vb also appear to be grouped in clusters rather than evenly distributed. Even though irradiation on E18 still produces periventricular ectopias, they contain no labeled neurons (Fig. 2C). There is a normal band of labeled neurons in layer Vb of animals irradiated on E18 after spinal cord injections. A second way in which the distribution pattern of corticospinal neurons in irradiated rats differs from normal is that displaced labeled neurons are located above Layer V in all irradiated animals (Fig. 2B–D). The scattered labeled neurons in the supragranular layers are found most frequently in places where the disruption of cortical lamination is the greatest. We have not examined the pattern of terminations of the projections of such abnormally located corticospinal neurons to determine whether the normal somatotopic organization has been disrupted.

Finally, there are changes in the dendritic morphology of individual labeled neurons in the irradiated animals. The primary dendrites of some neurons appear to be normally oriented towards the cortical surface, while in other neurons such dendrites are inverted or bifurcated. These alterations in dendritic morphology occur in labeled neurons located in the subcortical ectopias (Fig. 2C Inset) as well as in the labeled neurons of the infragranular layers (Fig. 2D Inset). The variety of dendritic alterations was greatest in animals irradiated on E17 and E18.

In summary, corticospinal neurons occur in two abnormal locations in adult rats that had been prenatally irradiated. In animals irradiated on E16 and E17, corticospinal neurons are located in periventricular ectopias, while all irradiated animals had at least a few corticospinal neurons scattered between layer V and the pial surface.

DISCUSSION

Prenatal irradiation produces corticospinal neurons in two abnormal positions. In animals irradiated on E16 and E17, corticospinal neurons occur in periventricular ectopias, while animals irradiated on E16–E19 have a few scattered corticospinal neurons in the supragranular layers. Thus, neurons located in abnormal positions in the adult telencephalon project to the spinal cord. We interpret this result as indicating that migration of the cell body to its usual adult location is not essential for neuronal processes to reach an appropriate target. Further, the finding that labeled neurons that project to the spinal cord are located in periventricular ectopias only when irradiation occurs on or before E17 suggests that the time at which a neuron is produced may be important in determining the target of that neuron. We base these interpretations on the following observations. The location of these ectopic corticospinal neurons, which are formed at the same time as layer V neurons are formed (see below) but situated below the white matter, indicates that their migration may have been prevented or interrupted. The interruption of migration has previously been suggested for similar ectopic formations (18, 19). Previous descriptions of recovery of the germinal neuroepithelium from prenatal X-irradiation indicate that the ectopias arise from small portions of neuroepithelium that have curled up and continued to proliferate (12, 14). The decreasing size of ectopias resulting from irradiation on successively later days is consistent with the decreasing proliferative activity of the neuroepithelium as development progresses (20, 21). We interpret the absence of labeled neurons that project to the spinal cord from ectopias after irradiation on E18 to indicate that neurons produced later than E17 do not normally project to this target. This conclusion is consistent with the known time of origin of cells in layer V (20, 21), the normal location of cells projecting to the spinal cord. An interpretation of the few supragranular corticospinal neurons is more complex because there are two reasonable alternatives. First, the corticospinal neurons of the supragranular layers may have been generated at a time corresponding to layer V neurons but passively displaced by subsequently formed neurons. This passive displacement could result from an alignment defect similar to that which has been suggested for neurons in the neocortex of the reeler mutant (22). A second alternative is that these supragranular corticospinal neurons are produced after E17 and migrate normally to the supragranular layers but are exposed to abnormal guidance cues, which direct their processes subcortically as they leave the germinal neuroepithelium. Cortical afferents present beneath the cortex on E16 (23, 24) may provide a substrate for growing efferent fibers. Cortical afferents are abnormally distributed in neocortex that has been altered prenatally (13, 25, 26) and consequently could function as an abnormal substrate for the growing processes of cortical projection neurons. A second point deserving emphasis is that the subcortical projections of the abnormally located corticospinal neurons persist into adulthood. This is of interest given that there are postnatal changes in the areal distribution of corticospinal neurons (4–6, 8). The abnormal position of corticospinal neurons in both the ectopias and the supragranular layers of the irradiated animals indicates that position is not a determining factor in maintaining such projections. While cell death does not appear to be involved in the developmental changes in the normal distribution of corticospinal neurons (8), an analogy can be drawn to the naturally occurring ectopic neurons in the avian isthmo-optic nucleus (ION) (many of which are eliminated by cell death). The changes in the distribution of ION neurons were initially related to cell location (27). More recent evidence, however, suggests that the major factor in determining the adult pattern of ION neurons may be the distribution of tectal afferents (28). By way of this analogy, thalamic projections in normal rats are primarily restricted to granular and infragranular layers. In contrast, when the number of neurons in the neocortex is reduced by prenatal insult, bundles of thalamic fibers extend into the upper layers (13, 26). Perhaps there are similar normal dendritic morphology of labeled neurons in a control animal. (Inset of C) Close-up of abnormal dendritic morphology of labeled neurons in the cortex of an animal irradiated on E18. (Scale bar = 0.1 mm and applies to all Insets.) (Inset of D) Close-up illustrating the abnormal orientation and unusual dendritic morphology of labeled ectopic neurons in an animal irradiated on E17. Note: Inset photographs are taken from different animals than represented in the larger photographs but from animals within the same irradiation groups.
changes in the distribution of the thalamic fibers in irradiated animals that play a role in maintaining the projections of ectopic corticospinal neurons. Such changes in the pattern of afferents may play a similar role in the irregularities in dendritic orientation that we have noted. This has been previously suggested to occur in the reeler mutant (29). Thus, the pattern of afferents may help direct initial growth and possibly contributes toward the selective maintenance of neuronal processes.

The present finding that ectopic neocortical neurons project to the spinal cord supports the conclusion based on observations of the neocortex of the reeler mutant mouse that neither a given migratory path nor a specific adult position is essential in establishing a particular subcortical projection (9, 10, 30, 31). The present study extends this conclusion by demonstrating that the initial projection of corticospinal neurons is determined very early in their individual ontogeny, indeed, prior to migration.

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