Connectivity of Ectopic Neurons in the Molecular Layer of the Somatosensory Cortex in Autoimmune Mice

Approximately 50% of New Zealand Black mice (NZB/BINJ) and 80% of NXSMD/EiJ mice prenatally develop neocortical layer I ectopias, mostly in somatosensory cortices. These cortical anomalies are similar to those seen in the brains of individuals with dyslexia. Neurofilament staining revealed a radial column of tightly packed fiber bundles in the layers underlying ectopias. This suggested that the connectivity of the ectopic neurons was aberrant. The present study used the tracers 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (Dil) and biotinylated dextran amine (BDA) to more thoroughly explore the cortical and thalamic connectivity of the ectopias. Dil placement into ectopias again revealed a distinct bundle of fibers extending from the ectopic neurons to the deep cortical layers. This bundle split in the white matter with some fibers traveling to the corpus callosum and others to the internal capsule. Thalamic connections were concentrated in the ventrobasal complex (VB) and posterior thalamic nucleus group (Po). Injections of BDA into VB revealed reciprocal connections between VB and the ectopic cortical neurons. Ipsilateral corticocortical projections were seen between ectopias in primary somatosensory and motor and secondary somatosensory cortices, but no contralateral connections of the ectopic neurons were seen. These findings confirm the notion that layer I ectopias are anomalously connected by comparison to neurons in homologous cortex, which may underlie widespread dysfunction of brains containing ectopias.

Introduction
The New Zealand Black (NZB/BINJ) and the recombinant inbred NXSMD/Ei mouse strains spontaneously develop ectopic collections of neurons (ectopias) in layer I of the neocortex (Sherman and Galaburda, 1985; Sherman et al., 1987a,b) that resemble those identified at autopsy in individuals with dyslexia (Galaburda and Kemper, 1979; Galaburda et al., 1985; Humphreys et al., 1990). Over 50% of these mice develop ectopias, usually with a single ectopia in the somatosensory cortex. The cortical anomalies are focal and contain neurons and glia in layer I with underlying disruptions in the lamination of layers II–VI. These ectopias form as early as E14–15 and are composed of neurons with birthdates that range from E13 to E18 (Sherman et al., 1992). Such a displacement of neurons probably results from a defect in the external limiting membrane, which normally prevents the migrating neurons from entering layer I (Caviness and Rakic, 1978). Mendelian breeding studies have suggested that the ectopia trait is related to autosomal recessive inheritance. Ectopias are also seen in fetal alcohol syndrome (Wisniewski et al., 1983) and in epilepsy (Meencke and Janz, 1984), but these differ in location and morphology to those seen in dyslexia.

There are changes in thalamic neuronal size in humans and rodents with cortical anomalies of this type (Livingston et al., 1991; Galaburda et al., 1994; Sherman et al., 1995; Jenner et al., 1996; Herman et al., 1997) that may also result from altered connectivity. We have hypothesized that the thalamic cell changes and connectional abnormalities associated with the ectopic neurons may relate to the learning difficulties seen in these mice (Denenberg et al., 1991a,b, 1992; Schrott et al., 1992, 1993; Boehm et al., 1996a,b; Waters et al., 1997) and in individuals with dyslexia. A critical step for supporting this view is to determine whether ectopic neurons are connected substantially with other cortical areas and with the thalamus. Preliminary findings showed that an antibody directed against the 68 kDa component of neurofilament protein revealed tightly packed, radially oriented fiber bundles within ectopias and in the underlying cortical layers (Sherman et al., 1990). Fibers from the bundles were also seen extending into the corpus callosum. The unusual organization of these fibers within the cortex, which were never present in mice without ectopias, indicated that the misplaced neurons might be aberrantly connected. It has not been determined, however, what are the specific targets of the neurofilament-labeled fibers, or whether the fibers originate from or terminate on the ectopic neurons. Therefore the tracers Dil (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) and BDA (biotin dextran amine) were used in the present study to determine whether, as expected, the ectopic neurons were connected abnormally to ipsilateral and contralateral cortices, as well as to thalamic nuclei.

Materials and Methods
Subjects
All mice were obtained from The Jackson Laboratories (Bar Harbor, ME). Seven adult NXSM-D (two female, five male) and four adult NZB/BINJ (one female, three male) fixed brains (see below) with single ectopias visible on the surface were used for the Dil studies. Ten ectopias were located in the primary somatosensory cortex (Par1) (Zilles, 1985), of which four showed incomplete diffusion of Dil. One ectopia was in the forelimb somatomotor cortex (FL). A separate group of adult NZB mice received small injections of BDA into the thalamus. Five mice from this group had both large ectopias in Par1 and accurate injections into the ventrobasal complex (VB). The remaining mice without ectopias were used for comparison purposes. Over 40 NXSM-D and NZB/BINJ brains without ectopias had Dil placed just under the pial surface in layer I of the homologous cortex. An additional 100 mice without ectopias were injected with BDA, from which animals with injection sites comparable to those in the experimental group were chosen for comparison.

Dil
Dil is a brightly fluorescent carbocyanine dye that diffuses in both the intercortical and retrograde directions labeling both afferent and efferent fibers from the site of Dil placement in either fixed or living tissue (Elberger and Honig, 1990; Haugland, 1996). Mice were anesthetized and transcardially perfused with 0.9% saline for one min followed by 2% paraformaldehyde/0.1% glutaraldehyde. The brains were removed and stored in this fixative. After several days the outside surface of the brain was lightly stained by immersion in a methylgreen/alcian blue solution. The brains were examined under a dissecting microscope to
locate ectopias. Large ectopias (∼300 µm in diameter) appeared as slightly raised small round protrusions on the surface of the brain. Using a glass micropipette with a diameter of 10–20 µm, a small crystal (∼10 µm in diameter) of DiI (Molecular Probes, Eugene, OR) was placed just under the pial surface onto the middle of the visualized ectopias. The DiI crystal usually was confined to layers I–II. In mice without ectopias a small crystal of DiI was placed just below the pial surface into the cortical areas that matched the location of ectopias in other brains. The brains were stored in fixative at 37°C for up to 4 months. The fixative was changed every 3–4 weeks.

The brains were cut with a Vibratome into 100 µm coronal sections and mounted on subbed slides. Most of the sections were viewed uncoverslipped with a Zeiss Axiophot microscope equipped with a rhodamine filter set (Zeiss set 15: BP 546, FT 580, LP 590) for viewing the orange–red DiI fluorescence. The remaining slides were coverslipped using a glycerol coverslip medium that contained 5% n-propylgallate to reduce photobleaching (Giloh and Sedat, 1982). Some sections were counterstained with m-phenylenediamine (m-PhD) so that cell bodies could be visualized (Quinn and Weder, 1988). This yellow–green background enhances the orange–red DiI signal.

![Figure 1](image1.png)

**Figure 1.** A computer digitized photomicrograph showing a typical injection site of BDA in VB (arrow). Although the injection sites varied, most were either circular or slightly oblong extending along the track of the pipette. The injection sites varied in size between 350 and 550 µm in diameter. Most, like this one, were located in the caudal part of the nucleus and usually included both VPM and VPL. Some of the larger injections extended into the lateral parts of Po or the medial part of Rt (bar = 100 µm).

**Table 1**

Summary of DiI results in both mice with and without somatosensory ectopias

<table>
<thead>
<tr>
<th>Bundle</th>
<th>Thalamus</th>
<th>Contralateral cortex</th>
<th>Ipsilateral cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ectopic Par1</td>
<td>no</td>
<td>VPM</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Po</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rt</td>
<td>*</td>
</tr>
<tr>
<td>Par1 ectopias</td>
<td>yes</td>
<td>VPM</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Po</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rt</td>
<td>*</td>
</tr>
<tr>
<td>Non-ectopic FL</td>
<td>no</td>
<td>VPL</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Po</td>
<td>****</td>
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<tr>
<td></td>
<td></td>
<td>Fr1</td>
<td>**</td>
</tr>
<tr>
<td>FL ectopia</td>
<td>yes</td>
<td>VPL</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Po</td>
<td>****</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fr1</td>
<td>****</td>
</tr>
</tbody>
</table>

*Relative intensity of DiI labeling.

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**Figure 2.** Digitized images showing an ectopia in the right Par1 cortex of an NXSM-D female. (A) A cluster of cells in layer I (arrows) in a coronal section stained with cresyl violet. (B) An adjacent section through the ectopia that was counterstained with the fluorescent Nissl stain m-phenylenediamine. This counterstain was used to confirm the location of DiI staining under the fluorescent microscope. (C) DiI staining of this ectopia is unlike the diffusion pattern in normal Par1 cortex (see Figure 2A for comparison). The DiI is confined to a narrow area in layer I (arrows). Bar = ∼200 µm.
fluorescent counterstain, which is illuminated using a FITC fluorescent filter set (Omega xF22: BP 485, FT 505, LP 530) allowed us to confirm that the DiI was accurately placed in ectopias and to identify the location of both thalamic and cortical connections.

BDA
Because of the fluorescent properties of DiI it is difficult to distinguish afferent and efferent connections as individual labeled cell bodies could not be visualized, therefore BDA was used to further characterize the connections between somatosensory cortical ectopias and the ventrobasal complex of the thalamus. BDA is a high molecular weight hydrophilic polysaccharide which in living tissue is actively transported in both the retrograde and anterograde directions (Haugland, 1996). BDA fills somas, dendritic arbors and synaptic terminals, giving labeled cells a Golgi-like appearance (Rajakumar et al., 1993).

Mice were anesthetized with an i.p. injection of Avertin (0.02 cc/g) and placed in a stereotaxic apparatus. BDA (10 kDa; Molecular Probes, Eugene, OR) was dissolved in distilled water (concentration of 10%) and was pressure injected using a glass micropipette (diameter 10–20 µm) attached to a Pneumatic PicoPump (World Precision Instruments, Inc., Sarasota, FL). With the force of two to three puffs of air at 20 psi for 5 ms, small amounts of BDA were injected bilaterally (to increase the likelihood of an ectopia being present) into the VB of the thalamus. The injection site, defined as that area where all cells were intensely and uniformly stained, varied in diameter between 350 and 550 µm (see Figure 1). In most animals, the injection site was centered in the caudal part of the VB and included both the ventral postero medial (VPM) and ventral posterolateral (VPL) thalamic nuclei. Larger injections sometimes included the lateral part of the posterior thalamic nuclear group (Po) and/or the medial part of reticular thalamic nucleus (Rt). The coordinates from the Franklin and Paxinos atlas (Franklin and Paxinos, 1997) were not accurate for NZB mice. Although the bregma–lambda distance measured by Franklin and Paxinos in C57BL/J6 mice (4.21 ± 0.51 mm) was similar in the NZB mice (4–5 mm), intersections of the lambdoid and bregma sutures through the sagittal suture were shifted caudally in NZB mice. In order to accommodate this shift the coordinates were set at AP –1.1, ML ±1.8 from the bregma and -5.2 from the dura.

Five days post-injection, the mice were anesthetized and perfused transcardially with 4% paraformaldehyde, then the brains were removed and cryoprotected in sucrose. The brains were then frozen and cut coronally at 30 µm on a sliding microtome. Free-floating sections were washed in PBS and incubated with avidin–biotin peroxidase complex at a concentration of 1 µl/ml in PBS for 1 h at room temperature. The sections were incubated in a freshly prepared solution of nickel enhanced diaminobenzidine tetrahydrochloride (DAB). The sections were mounted on subbed slides, counterstained with methyl-green/Alcian blue and coverslipped with Permount. Both anterograde and retrograde labeling were analyzed under a Zeiss light microscope.

In some mice, double labeling using an antibody (SMI-311 from Sternberger Monoclonal Inc., Lutheville, MD) directed against neurofilaments was performed to verify that a fiber bundle was present underlying an ectopia. The brain tissue was incubated overnight in the primary antibody at 4°C in a 1:1000 dilution. The vehicle (diluent) for all incubations was 5% rabbit serum in PBS. The sections were placed into a
solution containing the linking antibody (rabbit anti-mouse immunoglobulin; Dakopatts Z259 dilution 1/50) at room temperature for 2 h. The sections were exposed to a 1/250 dilution of mouse PAP (Dakopatts B650). The tissue was pre-incubated in a benzidine dihydrochloride (BDHC) solution without hydrogen peroxide for 10 min. The reaction was then carried out on ice to reduce background staining in freshly prepared BDHC solution which contained 0.005% hydrogen peroxide. The tissue was processed for BDA as above except that non-nickel-enhanced DAB was used so that the DAB would not develop too darkly and obscure the other label. Adjacent sections of tissue were processed using the neuronal nuclei antibody (NeuN) which specifically labels neuron-specific nuclear protein, to confirm that the BDA-labeled cell bodies in the ectopias were neurons (Mullen et al., 1992). The NeuN antibody protocol was the same as that described for the double labeling of neurofilament and BDA described above. The tissue was incubated overnight at 4°C in a 1/500 dilution NeuN antibody (Chemicon International Inc. Temecula, CA). The vehicle (diluent) for all incubations was 5% horse serum in PBS. The linking antibody for NeuN was a horse anti-mouse immunoglobulin.

Results

NZB and NXSM-D mice with and without cerebrocortical ectopias in Par1 and FL were examined to determine how the connectivity of cortex with ectopias differs from developmentally normal cortex. No strain or sex differences were noted, so the analyses were collapsed over these variables. Results of all Dil experiments are summarized in Table 1. The ectopia in FL differed slightly in its connectivity to those located in Par1 and is described below.

Comparisons between the patterns of Dil labeling around the site of crystal placement differed substantially between cortex with and without ectopias. When Dil was placed within an ectopia most labeling was confined within the boundaries of the ectopia. This resulted in a dense cone-shaped pattern of diffusion at the center of the ectopia that narrowed in the upper part of layer II (Figure 2C). Dil label was less confined in non-ectopic cortex, which characteristically showed a substantial amount of lateral spread surrounding the crystal placement (Figure 3A). The average lateral spread from Dil placed in ectopias was ~1 mm whereas the average lateral spread from Dil placed in normal cortex was ~2 mm.

Underlying the ectopias was a distinct radial bundle of labeled fibers (Figure 4) identical to those visualized with immunohistochemical staining of neurofilament protein (Sherman et al., 1990). In the mice without ectopias, labeling under the Dil crystal revealed no fiber bundles. Instead labeling was seen in all cortical layers and was restricted in layer IV, producing a distinct hourglass shape (Figure 3A). In parts of the barrelfield Par1 fibers extended through layer IV in narrow fascicles between the barrels and individual fibers could be seen extending into the white matter (Figure 3B).
internal capsule and the other in the corpus callosum. Fibers in the internal capsule connected to subcortical nuclei. Although some fibers were visible ventrally, most entered the thalamus through the reticular nucleus (Rt), where in a few cases there was distinct labeling. Extensive labeling, however, was restricted to the VPM division of VB (Figure 5A). Fifty percent of the cases with ectopies in Par1 also had light labeling in Po (Figure 5B). The ectopia in Fl on the other hand connected exclusively with the VPL division (Figure 5C). This staining pattern differed from the control cases, where individual fibers in the internal capsule resulted in a greater intensity of staining in Po than in VB, a known pattern of connectivity of the supragranular layers (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991; Diamond et al., 1991, 1992; Guillery, 1995; Sherman and Guillery, 1996). Because of the intensity of fluorescence from Dil, retrogradely labeled cell bodies could not be distinguished from anterogradely labeled fibers.

In brains with ectopies, the Dil-labeled fibers in the corpus callosum crossed the midline, but no labeling was visible in the contralateral cortex or subcortical regions. This was in contrast to the labeling of the non-ectopic cortex, which strongly labeled areas homologous to the Dil placement in the contralateral cortex. Thus, in mice without ectopies, there was a light but distinct column of Dil-labeled cortex, with labeled neuronal bodies in layers II and IV, and radially oriented fibers throughout the cortical layers (Figure 3C).

Despite the lack of contralateral corticocortical connections to and from ectopies, Dil placed onto ectopies did show the presence of ipsilateral corticocortical connections. For instance, labeled cell bodies and fiber terminals were seen in the supragranular layers of the ipsilateral primary motor cortex (Fr1) (Figure 6A,B). In addition, fibers, but no retrogradely labeled cell bodies, were seen in the supragranular layers of lateral parts of Par1 and the secondary somatosensory cortex (Par2) (Figure 6C,D). Although a similar pattern of staining was seen in normal mice, the intensity of the labeling was reduced in both the motor and somatosensory cortices.

BDA injections in VB were used to more accurately delineate the connections between ectopies and VB. Results of BDA injections in both mice with and without ectopies are summarized in Table 2. BDA injections into VB revealed only a few labeled fibers within the bundles underlying ectopies. The presence of fiber bundles in these brains was verified by double-labeling for neurofilament. A dense plexus of BDA-positive fibers confined within the boundaries of the ectopia, however, was seen. These fibers had a punctate appearance suggesting that they had numerous synaptic terminations with the ectopic neurons (Figure 7). In addition to this fiber network, BDA-labeled cells that stained positively for NeuN were visualized within the ectopies. In one case, a few anomalous labeled cell bodies were also seen in layers III and IV (Figure 7). Also, a large number of thalamocortical terminals in layer IV and pyramidal-shaped cell bodies in the infragranular layers were labeled. Beneath the ectopies themselves the density of labeled fibers and neurons was less than in the surrounding cortex.

In non-ectopic cortex, labeled cell bodies were never seen in layer I, but were confined to the infragranular layers. Only a small number of fibers were seen entering layer I. These fibers transected the cortex perpendicular to the pial surface with limited branching in layer I. Laminar staining was consistent with that previously reported, with the majority of anterogradely-labeled terminals located in layer IV and retrograde-labeled neurons in layers V and VI (Emmers, 1988; Armstrong-James and

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Figure 5. Digitized images showing labeling in the thalamus from DiI placed in the center of ectopies in NXS-M and NZB mice. (A) The labeling in the right VPM (arrow). (B) Staining in both VPM and Po. The arrows mark the approximate border between these two nuclei. (C) Intense labeling in VPL (arrow) from an ectopia in the left FL. Bar = 100 µm.
Discussion

NZB and NXSM-D mice without ectopias show the typical pattern of thalamic and cortical connectivity of the somatosensory cortex. Use of the tracers DiI and BDA revealed that Par1 was reciprocally connected with the ipsilateral primary motor cortex (Fr1) and projected laterally to Par1 and Par2. Further, reciprocal connections were seen arranged in a columnar fashion in the contralateral somatosensory cortex, and were also seen subcortically with thalamic nuclei VB and Po.

In NZB and NXSM-D mice with molecular layer ectopias, however, the organization of the connections of the somatosensory cortex was unusual. First, as visualized with neurofilament antibodies (Sherman et al., 1990), there was a dense, radially oriented bundle of fibers underlying the ectopias which BDA labeling revealed contained both afferent and efferent fibers. It appeared as if this bundle resulted from a bottleneck at the lower edge of the ectopias. Electron microscopic analyses of cortical ectopias have revealed thin pericyte septae encircling the ectopias (Boehm et al., 1995). These septae may act as a boundary separating ectopias from the adjacent cortex, restricting the path by which fibers enter and exit the ectopias. Thus bundles appear to be an important characteristic of the anatomical organization of cortex that contains an ectopia. Additionally, the confinement of the BDA-labeled fibers to the ectopias also suggests that this boundary prevents the labeled fibers from extending into the surrounding cortex and may be responsible for reducing the lateral spread of Dil placed into an ectopia, resulting in a cone of intensive labeling and less lateral spread of the Dil than seen in non-ectopic cortex.

We thought that the bundles, although resulting from structural alterations at the site of ectopias, were accompanied by a more widespread disorganization of the connectional architecture; therefore, we examined the termination and origin of the fibers in these bundles. Fibers in the bundles were connected both cortically and subcortically. Axons originating in the ectopias that extended subcortically could be visualized in the internal capsule and thalamus, where they connected with nuclei Rt, VB and Po. No other thalamic nuclei were labeled. The locations of these connections correspond to those seen in mice without ectopias but with different intensity of staining. In mice with ectopias staining was more robust in VB, with only light staining seen in Po. In mice without ectopias this pattern was reversed, with greater staining in Po than in VB. This shift in intensity may be due to the heterogeneous population of neurons in ectopias. Birthdating studies have shown that ectopias...
consist of neurons from all cortical layers (Sherman et al., 1992). Normally the thalamocortical projections from VB terminate in layer IV, whereas the majority of corticothalamic projections originate from layer VI of the somatosensory cortex (Emmers, 1988; Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991; Diamond et al., 1991, 1992). Displaced layer IV and VI neurons in ectopias may be responsible for the robust staining in VB. In normal cortex these neurons are not labeled by the superficial placement of DiI, which only labels layer I. Layer I has been shown to have numerous connections with Po and sparse connections with VB (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991; Diamond et al., 1991, 1992; Guillery, 1995; Sherman and Guillery, 1996). This pattern of connections, which was confirmed by the BDA injections into VB, would result in the robust staining in Po and light staining in VB visualized in mice without ectopias. Further, the reduction of Po labeling from the ectopias may reflect the lack of DiI spread outside the ectopia, which would result in less layer I labeling. Further studies combining birthdating and tracers must be done to confirm this hypothesis.

Analysis of the cortical connectivity of the ectopias revealed labeled fibers from ectopias to ipsilateral cortical areas. Both retrograde and anterograde labeling were seen ipsilaterally in Fr1 and anterograde labeling was seen in lateral parts of Par1 and Par2. Like the thalamus, the ipsilateral cortical connections from ectopias do not differ in location, but rather in intensity from those connections seen in non-ectopic cortex — in normal cortex staining was not as dense as that seen in mice with ectopias. This intensity difference, like that in the thalamus, may also reflect the heterogeneous population of neurons labeled within the ectopias. Corticocortical projections normally originate from layers II/III and V (Killackey et al., 1989; Koralek et al., 1990; Catalano et al., 1991). Typically, only those neurons in the upper part of layer II would be labeled with DiI, labeling only a small

| Table 2 | Location of BDA labeling in Par1 cortex in mice with and without ectopias |
|---------|-----------------|-----------------|
| Cortical layers | Retrograde cell | Anterograde fibers |
| I        | ****           | ****            |
| II       | **             | **              |
| III      |                |                 |
| IV       | *              | ***             |
| V        | ***            |                 |
| VI       | **             | **              |
| Normal Par1 |                |                 |
| I        | *              | **              |
| II/III   |                | **              |
| IV       | ****           |                 |
| V        |                | **              |
| VI       |                | **              |

*Relative intensity of BDA labeling.

Figure 7. Digitized images showing a large ectopia in the right Par1 cortex labeled with BDA. Retrograde-labeled cell bodies and anterograde-labeled fibers are seen in layer I. This pattern differs from that seen in non-ectopic Par1 cortex. The normal pattern of connections was present in the surrounding cortex. In addition, there were anomalous connections with the cortex underlying the ectopia. There were a number of retrograde-labeled cell bodies in both layers II and IV (arrows), suggesting that these cells send axons to VB. Retrograde-labeled cells were never seen in the superficial layers in normal Par1 cortex (not shown). (A) The outlined area in panel A at a higher magnification. This digitized image show those BDA labeled neurons in the supragranular layers underlying the cortex underlying an ectopia in somatosensory cortex. Bar (A) = 100 µm, (B) = 10 µm.
portion of the neurons responsible for these connections. However, when Dil is placed within the heterogeneous cells of the ectopias it might label a greater proportion of the ipsilateral connections, resulting in greater staining. With this argument in mind one could hypothesize that contralateral connections should be greater from ectopias than normal cortex; however, the opposite result was obtained.

Dil-labeled fibers from the bundles were seen in the corpus callosum and appeared to cross into the opposite hemisphere. There was no evidence, however, that they were connected with neurons in the contralateral cortex. This differed from the clear contralateral connections seen in the cortex of mice without ectopias. This could not be explained by diffusion distance or time. The distance to labeled thalamic targets was as great as the distance to non-labeled contralateral cortical targets, and there was no lack of contralateral cortical labeling in controls after the same diffusion time. The most likely explanation for the lack of clear contralateral staining in mice with ectopias is that the contralateral cortical connections were greatly reduced and so diffuse that they could not be seen above the background fluorescence.

Similar populations of neurons in layers II/III and V give rise to both the contralateral and ipsilateral cortical connections. The present results suggest a reduction in the former and an increase in the latter. It is plausible that neurons from these cortical layers, when displaced to the ectopias, connect ipsilaterally rather than contralaterally. Because there is competition for cortical targets between thalamic and transcallosal neurons (Ivy et al., 1979; Ivy and Killackey, 1981; Innocenti and Clarke, 1984; Finlay et al., 1986; O’Leary and Stanfield, 1989), the lack of contralateral connections of the ectopic neurons may result in greater connections with ipsilateral cortical areas, as reported here, and with thalamic nuclei. Further analyses may reveal a quantitative difference in both the cortical and thalamic connections in mice with ectopias, providing additional evidence of the altered anatomical organization of these animals.

Differences in connectivity like those reported here may contribute to the changes in neuron size seen in the thalamus of humans and rodents with early cortical malformation (Livingstone et al., 1991; Galaburda et al., 1994; Sherman et al., 1995; Jenner et al., 1996; Herman et al., 1997). It has been hypothesized that these thalamic changes result from the top-down effects of early cortical ectopias. Our tracer studies show that the ectopic neurons are connected with the thalamus, thus providing a pathway for thalamic changes. Changes in thalamic cell size and the alteration of contralateral cortical connections may play a role in the learning differences seen in mice and humans with molecular layer ectopias (Boehm et al., 1996a,b; Denenberg et al., 1991a,b; Schrott et al., 1992, 1993; Waters et al., 1997).

**Notes**

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