Introduction

In humans, cortical dysgenesis occurs in a variety of congenital brain anomalies, with a deviation from normal development during the formation of the cerebral cortex as common etiology. The cerebral cortex is formed by the proper completion of a number of complex processes that take place in the second half of gestation and extend into the postnatal period. These processes include neurogenesis and gliogenesis, neuronal migration, neuronal cellular commitment, neurite outgrowth and axonal pathfinding, formation of synapses and naturally occurring cell death (Rakic et al., 1994; Rakic, 1995; Rakic and Lombruso, 1998; Kopper, 1998; Haydar et al., 1999). It is likely that each of these processes can be implicated in some form of cortical dysgenesis (Rorke, 1994). However, the histological changes seen in many forms of cortical dysgenesis, such as lissencephaly, polymicrogyria, schizencephaly, or laminar heterotopia, suggest that at least part of their pathogenesis affects the normal migration of immature cortical neurons. Therefore, these conditions have also been described as neuronal migration disorders (Roper, 1998). A number of genes have been identified that participate in the regulation of neuronal migration (Copp and Harding, 1999; Rubenstein and Rakic, 1999; Gleeson and Walsh, 2000; ten Donkelaar, 2000; Ross and Walsh, 2001). In fact, some forms of neuronal migration disorders, such as lissencephaly type I or bilateral periventricular nodular heterotopia, have a genetic etiology (Dobyns et al., 1993, 1996). However, in a large number of sporadic cases of neuronal migration disorders, the etiology is unknown (Roper, 1998). A possible role of environmental factors was suggested by earlier workers (Palmini et al., 1994), who found that prenatal potentially harmful environmental events, such as significant maternal physical trauma, infections or X-ray examinations, may play a central role in the pathogenesis of neuronal migration disorders in humans. Accordingly, several injury-based animal models have been developed to determine the mechanisms underlying the development of disturbed neuronal migration, including in utero irradiation, in utero exposure to ethanol and perinatal cortical freeze lesions (Roper, 1998). Alterations of neuronal migration following in utero irradiation have been studied by injecting pregnant rodents with bromodeoxyuridine (BrdU) at different days of gestation, followed by whole-body exposure to ionizing radiation with dosages ranging from 10 to 250 cGy. The distribution patterns of BrdU-labeled cells were then analyzed by immunohistochemistry at different pre- and postnatal time points (Inouye et al., 1993, 2000; Sun et al., 1996, 1999; Fushiki et al., 1997; Hyodo-Taguchi et al., 1997). However, such approaches suffer from several methodological limitations. First, the analysis is restricted to the pattern of the BrdU-labeled cells and thus to a small subpopulation of cells whose progenitors were in S-phase during the injection of BrdU. Second, it cannot be excluded that the injection procedure itself contributes to alterations of neuronal migration, since injections into pregnant rodents have been shown to alter the physiologic development of the immature brain, including neuronal loss (Poland et al., 1994; Cratty et al., 1995; Schmitz et al., 1999b). Third, the results of these studies may be biased by a loss of BrdU-containing cells, since the incorporation of BrdU into the DNA of mammalian cells may cause them to become more sensitive to the deleterious effects of ionizing radiation (Djordjevic and Szybalski, 1960; Iliakis et al., 1989; Hawkins, 2001). Fourth, it is difficult to compare the results of these studies to histological changes seen in neuronal migration disorders in humans, as it is strictly impossible to label cells in the developing human cerebral cortex with BrdU.

To circumvent these limitations, we developed a novel method to investigate the three-dimensional (3D) spatial arrangement of neurons in thick sections without use of BrdU or any other pretreatment. Based on our recent finding of a 24% reduction in the volume of mouse layer V without loss of pyramidal cells in this layer in a model of prenatal low-dose X-irradiation (Korr et al., 2001), we have analyzed the repercussions of subjecting pregnant mice to a single whole-body X-irradiation with 50 cGy on day 13 of gestation on the 3D spatial arrangement of layer V pyramidal cells in the brains of their offspring.

Materials and Methods

Animals

Twelve timed pregnant Han:NMRI mice were kept under specific...
pathogen-free conditions at the Institut für Versuchstierkunde, RWTH University of Aachen, Germany (four animals per cage, air-conditioned rooms, 20°C, 60% humidity, 12:12 h light:dark cycle with artificial lights on at 6:00 a.m., ad libitum access to water and Altromin standard diet). At day 12 of gestation (E12, where E is the day after overnight mating), animals were brought to the Department of Radiobiology of the Belgian Nuclear Research Centre (CEN-SCK, Mol, Belgium). On the following morning (E13), each pregnant mouse was immobilized using a Plexiglas tube and whole-body X-irradiation was carried out with 50 cGy under the following conditions: 250 kV X-rays filtered with 1 mm Cu at a dose-rate of 590 mGy/min. Doses were determined by a Farmer ionization chamber (Reyners et al., 1992). The period of immobilization was 7 min for all animals. Following X-irradiation, the mice were kept in individual cages. Sham-irradiated animals were manipulated in a similar way, but were not irradiated. No differences in health, activity pattern and maintenance of a normal diet schedule were observed between irradiated and sham-irradiated animals. Birth of pups (=P1) took place at E18 or E19, with no difference in mean litter size between irradiated and sham-irradiated animals. Birth of pups (≥P1) took place at E18 or E19, and weaning took place at P25. All investigations were conducted in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1987).

**Histological Procedures**

On P180, one male offspring per dam was anesthetized with chloral hydrate (10% aqueous solution, 0.005 ml/g body wt, i.p.) and was killed 5 min later by intracardial perfusion fixation with formalin solutions as previously described (Korr et al., 1989; Schmitz et al., 1999a). The heads of the animals were fixed in formalin solution for 24 h at 4°C. Afterwards the brains were removed, halved in the medio-sagittal line and further fixed for 10 days. The right brain halves were cryoprotected in sucrose solution (10, 20 and finally 30% sucrose in 0.1 M Tris–HCl buffer, 2 × 12 h per solution; 4°C) and were frozen in Tissue-Tek® (Sakura Finetek Europe, Zoeterwoude, The Netherlands). The brain halves were then entirely cut to serial, 150 µm thick frontal sections. Every second section was selected with a random start, mounted, dried, defatted with Triton X-100 (0.025%, 20 min; Merck, Darmstadt, Germany) and stained with gallocyanin (Heinsen and Heinsen, 1991). All slides were covered with a coverslip using DePeX® (Serva, Heidelberg, Germany).

**Stereological Analysis**

For each animal, the volume of layer V was investigated using Cavalieri's principle (Cavallieri, 1635) considering possible overprojection (Gundersen and Jensen, 1987), as well as the total number of layer V pyramidal cells using the optical fractionator principle (Gundersen, 1986; Gundersen et al., 1988; West et al., 1991; Schmitz, 1998; Schmitz and Hof, 2000) and the number-weighted mean perikaryal volume of the pyramidal cells using the nucleator principle (Gundersen, 1988). Based on these data, the neuronal density in layer V was calculated for each animal (by dividing the total number of layer V pyramidal cells by the volume of this layer), as well as the percentage of the volume of layer V occupied by pyramidal cell perikarya (by dividing the product of the mean perikaryal volume of the pyramidal cells and the total number of these cells by the volume of layer V).

During the cell counting procedure to investigate the total number of layer V pyramidal cells, the \(x-y-z\) coordinates of the nuclear center were recorded for each registered neuron (‘parent neuron’). To find the nearest neighbor to this ‘parent neuron’, the surrounding tissue was entirely scanned in all directions \(x\), \(y\) and \(z\), starting in the immediate vicinity around the ‘parent neuron’. With this procedure, which guaranteed that the nearest neighbor could not be missed, all pyramidal cells next to the ‘parent neuron’ were located (‘offspring neurons’) and the \(x-y-z\) coordinates of the nuclear center of these ‘offspring neurons’ were recorded. Based on these data, the distance between each ‘parent neuron’ and the corresponding nearest ‘offspring neuron’ (i.e. the NND) was determined. Finally, all NNDs of a given animal were used to calculate the NND distribution of this animal.

To compare the obtained NND distributions with theoretical NND distributions based on the same total number of neurons and the same neuronal density as estimated in the stereological investigations, appropriate computer simulations were performed following published recommendations (Diggle, 1983).

The on-line supplementary material to this study provides a thorough description of the new stereological procedure carried out in this study, as well as the basic ideas underlying the computer simulations.

**Statistical Analysis**

For each group, the mean and the standard error of the mean were calculated for all of the investigated variables (i.e. the volume of layer V, the total number of layer V pyramidal cells, the neuronal density in this layer, the layer, the prenatally irradiated animals, the percentage of the volume of layer V occupied by pyramidal cell perikarya, the minimum, mean and maximum NND, as well as the 25th percentile, the median and the 75th percentile of the NND distributions). Comparisons between the groups were performed with the Student’s two-tailed \(t\)-test. Statistical significance was established at \(P < 0.05\). All calculations were performed using GraphPad Prism (v. 3.00 for WindowsSM; GraphPad Software, San Diego, CA).

**Graphical Analysis**

To obtain information regarding the type of spatial arrangement of the pyramidal cells within layer V (i.e. random, dispersed, or clustered spatial arrangement), the cumulated relative frequency distribution of the NNDs was compared to the corresponding distributions obtained from the computer simulations in each animal. This was performed by graphical analysis using empirical distribution function (EDF) plots as described previously (Diggle, 1983). A brief introduction to the analysis of NND distributions by EDF plots is also provided in the on-line supplementary material to this study.

**Results**

A significantly smaller mean volume of layer V was found for the prenatally irradiated animals compared to the sham-irradiated ones. Both groups of animals showed almost the same mean total number of layer V pyramidal cells, with very similar mean perikaryal volumes. The mean neuronal density in layer V of the prenatally irradiated animals was increased compared to the sham-irradiated animals, albeit not statistically significantly. In the prenatally irradiated animals, on average a higher percentage of the volume of layer V was occupied by pyramidal cell perikarya than in the sham-irradiated animals, albeit not reaching statistical significance (upper part of Table 1).

The individual box-and-whisker plots of the NND distributions for layer V pyramidal cells showed differences between the prenatally irradiated and the sham-irradiated animals (Fig. 1). For each prenatally irradiated animal, the median of the NND distributions was >20 µm (lower stippled lines in Fig. 1). In contrast, for three out of the six sham-irradiated animals, the median of the NND distributions was <20 µm (animals nos 9, 10 and 12). Furthermore, for four out of the six prenatally irradiated animals the greatest NND was >60 µm (animals nos 1, 2, 5 and 6; upper stippled lines in Fig. 1), whereas for all sham-irradiated animals the greatest NND was ≤60 µm. There was no statistically significant difference between prenatally irradiated and sham-irradiated animals with respect to the averages of the minimum NND and the 25th percentiles of the NND distributions. In contrast, the averages of the medians of the NND distributions, of the mean NNDs, of the 75th percentiles of the NND distributions and of the maximum NNDs were significantly greater for the prenatally irradiated animals when compared to the sham-irradiated animals (lower part of Table 1). These differences between the prenatally irradiated and the sham-irradiated animals were also the basis for the different averaged cumulated relative frequency distributions of the NNDs for layer V pyramidal cells. In the graphical representation, the averaged cumulated relative frequency distribution of the NNDs for layer V...
V pyramidal cells of the prenatally irradiated animals was shifted to the right compared to the corresponding distribution of the sham-irradiated animals (Fig. 2). For example, in sham-irradiated animals ∼55% of the NNDs were 20 µm or smaller, whereas in prenatally irradiated animals only ∼35% of the NNDs were of this size (arrows in Fig. 2).

Additional insight into differences between the prenatally irradiated and the sham-irradiated animals was obtained from the EDF plots of the NNDs for layer V pyramidal cells (Fig. 3). For all animals, the EDF plots of the NNDs were found outside the simulated envelopes. All EDF plots were shifted to the right when compared to the simulated envelopes. However, this was more pronounced for the prenatally irradiated animals than for the sham-irradiated animals. In consequence, complete spatial randomness of the 3D arrangement of the pyramidal cells in layer V was not observed in any animal. Rather, the EDF plots pointed to a deficiency of small NNDs (as explained in detail in the on-line supplementary material to this study), which was more pronounced in the prenatally irradiated animals than in the sham-irradiated ones.

Discussion

This study provides the first quantitative analysis of the 3D spatial arrangement of neurons in the adult mouse brain following prenatal low-dose X-irradiation by investigating NND distributions. Subjecting pregnant mice to a single whole-body X-irradiation with 50 cGy on day 13 of gestation caused a reduction of the mean volume of layer V by ∼26% compared to sham-irradiated controls. However, no loss of layer V pyramidal cells and no changes in the mean perikaryal volumes of these cells were observed in irradiated animals. Layer V pyramidal cells showed a more dispersed spatial arrangement in irradiated than

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prenatally irradiated animals</th>
<th>Sham-irradiated animals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of cortical layer V (mm³)</td>
<td>0.73 ± 0.05</td>
<td>0.99 ± 0.07</td>
<td>0.014</td>
</tr>
<tr>
<td>Total number of layer V pyramidal cells</td>
<td>59 795 ± 50044</td>
<td>62 194 ± 7118</td>
<td>0.639</td>
</tr>
<tr>
<td>Mean perikaryal volume of pyramidal cells</td>
<td>4847 ± 137</td>
<td>5043 ± 151</td>
<td>0.359</td>
</tr>
<tr>
<td>Neuronal density in layer V (&gt;1/mm³)</td>
<td>81 249 ± 9460</td>
<td>64 138 ± 8482</td>
<td>0.208</td>
</tr>
<tr>
<td>Percentage of the volume of layer V occupied by pyramidal cell perikarya (%)</td>
<td>39.6 ± 4.93</td>
<td>31.9 ± 3.64</td>
<td>0.237</td>
</tr>
</tbody>
</table>

P-values of Student’s two-tailed t-test; those in bold indicate statistical significance. NND, nearest neighbor distance.

Figure 1. Individual box-and-whisker plots of the NND distributions for layer V pyramidal cells of prenatally irradiated animals (nos 1–6) and sham-irradiated animals (nos 7–12). Lower stippled lines, median data; upper stippled lines, maximum data.

Figure 2. Graphical representation of the averaged cumulated relative frequency distributions of the NNDs for layer V pyramidal cells of prenatally irradiated animals (50 cGy) and sham-irradiated controls (0 cGy).

Figure 3. Individual empirical distribution function plots of the NNDs for layer V pyramidal cells of prenatally irradiated animals (nos 1–6) and sham-irradiated controls (nos 7–12), with upper and lower envelopes from each of the 99 runs of the computer simulations (dotted lines).
in sham-irradiated animals. The mean layer V volumes and mean numbers of layer V pyramidal cells from the present study are very similar to our recent data on the same animals using a different stereological sampling scheme (Korr et al., 2001). In that study, we used optical dissectors with a base area of 1845 µm² (versus 3671 µm² in the present study) and height of 20 µm (versus 6 µm), stepping distances between the optical dissectors in the x and y directions of 150 µm (versus 120 µm) and the unit of counting was the top of the nucleus of the pyramidal cells (versus the center of the nucleus of the pyramidal cells). This sampling scheme could not be applied in the present study since the use of 20 µm thick optical dissectors would not have guaranteed registering of cells in the middle of the section thickness. However, registering of cells in the middle of the section thickness was the requirement to find the nearest neighbor of the corresponding ‘parent neuron’ within the section thickness (an in-depth discussion of methodological aspects concerning the analysis of the spatial arrangement of neurons in a given brain region by NND distributions and empirical distribution function plots is provided in the on-line supplementary material to this study). In our earlier study (Korr et al., 2001), layer V volumes were 0.75 ± 0.06 mm³ (mean ± SD) in irradiated animals and 0.98 ± 0.08 mm³ in sham-irradiated animals. The numbers of layer V pyramidal cells were 59 821 ± 10 037 in irradiated animals and 62 168 ± 5633 in sham-irradiated animals. These data are fully comparable to those reported in the present analysis.

The findings of the present study indicate that subjecting pregnant mice to a single whole-body X-irradiation with 50 cGy on day 13 of gestation results in a more dispersed spatial arrangement of layer V pyramidal cells of their adult offspring. This difference in spatial arrangement coincides with a reduction of layer V volume by ∼26% but no loss of neurons compared to controls. This accentuated dispersal of the same number of neurons within a reduced reference space (i.e. layer V) is possible because only ∼30% of the volume of layer V is occupied by pyramidal cell perikarya in the brains of the controls (Table 1). Due to the estimation of mean perikaryal size with the nucleator method applied on coronal sections, the absolute value of ∼30% can only be considered as a relatively rough estimate (Schmitz et al., 1999c). However, these data clearly indicate that most of the volume of layer V did not contain perikarya of pyramidal cells, which permits a more dispersed spatial arrangement of the cells following prenatal irradiation, even if the reference space containing the cells was reduced.

Let us consider briefly the potential biological significance of a more dispersed spatial arrangement of the cells potentially caused by alterations of neuronal migration, and a reduced reference space containing the cells pointing to a reduced neuropil. The fetal stage most susceptible to the induction of histogenetic abnormalities such as dysgenetic hydrocephaly, microcephaly, heterotopia of cortical gray matter, abnormal cortical architecture and defects of corpus callosum by external agents, corresponds to the beginning state of neuron differentiation from matrix cells in both human and experimental animals such as mice and rats (Hoshino, 1983). With respect to low-dose X-irradiation, the highest sensitivity was shown in mouse matrix cells of the telenencephalon in day 13 of gestation (Kameyama, 1983). In this stage, young neurons begin to differentiate from the proliferating germinal cells (Hoshino, 1983). According to Shimada et al. (Shimada et al., 1977), progenitors of layer V pyramidal cells, but also of cerebellar Purkinje cells, become postmitotic in the developing mouse brain on day 13 of gestation and begin to migrate and to differentiate at this time point. We have recently demonstrated that the prenatal X-irradiation paradigm used here (i.e. subjecting pregnant mice to a single whole-body X-irradiation with 50 cGy on day 13 of gestation) also resulted in a loss of cerebellar Purkinje cells by ∼21% (Schmitz et al., 2000). This is consistent with the results of similarly designed studies in the literature (Ralcewicz and Persault, 1994, 1995).

The findings presented here agree with data from previous studies on morphological abnormalities in the cerebral cortex of rodents prenatally exposed to higher doses of X-irradiation. For example, Fukui et al. (Fukui et al., 1991) exposed pregnant rats to a single whole-body gamma-irradiation on day 15 of gestation — which roughly corresponds to day 13 of gestation of mice with respect to the development of the central nervous system (Hoshino and Kameyama, 1988) — with doses of 27, 48, 100 or 146 cGy. Investigating adult offspring, the authors found a reduction in cortical thickness in the groups exposed to 48 cGy or more, with an increase in cell packing density up to the group exposed to 100 cGy. Similar to the present findings, these results indicate an increased overall cell packing density, but no loss of neurons following prenatal X-irradiation at a dose of ∼50 cGy. In another study (Sun et al., 1999), pregnant mice were subjected to 150 cGy of X-irradiation on embryonic day 14. In 4-week-old prenatally irradiated offspring, the authors found a thinner layer V than in sham-irradiated controls, as well as a dispersion of large pyramidal cells in layers II–V. Furthermore, they applied immunohistochemistry to investigate midkine-stained radial glial fibers in the brains of fetal offspring. Midkine is a heparin-binding growth factor specified by a gene responsive to retinoic acid. Increased expression of midkine was detected on the processes of radial glial cells in the developing rat cerebral cortex (Matsumoto et al., 1994). Using this technique, Sun et al. (Sun et al., 1999) found the radial glial fibers crumpled in the lateral region of the brain and markedly reduced in number in some parts of the dorsal region of the brain following prenatal 150 cGy X-irradiation. In brains of postnatal offspring, fibers of radial glial cells stained with anti-GFAP antibody were disorganized following prenatal X-irradiation when compared to sham-irradiated control animals. The authors concluded that the disturbed migration of cortical neurons following prenatal X-irradiation — with the same tendency towards a more dispersed spatial arrangement as found in the present study — was mainly based on the destruction of neuronal migratory pathways (radial glial fibers) in the developing cerebral cortex.

Several authors have reported disturbances of initial neuronal migration in the fetal brain following prenatal low-dose X-irradiation, with compensatory mechanisms during the postnatal period. For example, Pusshiki et al. (Pusshiki et al., 1997) labeled proliferating cells on day 14 of gestation of mice with BrdU followed by a single exposure to 10–100 cGy of X-rays and investigated the brains of embryos on day 17 and of offspring at 2, 3 and 8 weeks after birth. By analyzing camera lucida drawings and layer-specific counts of BrdU-positive cell profiles on selected sections, the authors showed that the initial migration of BrdU-labeled cells from the matrix cell zone towards the cortical plate during embryonic periods was decelerated when the animals were prenatally exposed to X-rays of 25, 50 and 100 cGy. Furthermore, abnormally distributed neurons in the cerebral cortex were noted in young animals that were irradiated prenatally but not in old ones. Hyodo-Taguchi et al. (Hyodo-Taguchi et al., 1997) injected pregnant mice with BrdU on day 14 of gestation and exposed the animals to 153Cs gamma rays continuously for 3 days at dose rates of 10, 30 and 94 cGy/day. Also by analyzing camera lucida drawings and
On the other hand, neuronal densities in the entorhinal cortex have interpreted this finding as being compatible with a disturbance of neuronal migration during brain development. However, in 8-week-old mice no difference was found in the distribution pattern of BrdU-labeled cells between control animals and those irradiated with 10 and 30 cGy/day. Considering the new results presented here, it is possible that the lack of difference in distribution patterns in adult animals in these studies (Fushiki et al., 1997; Hyodo-Taguchi et al., 1997) was due to less rigorous methods employed by these authors. The distribution of cells within a given region of the brain may have important implications for the function of that region, in that the ability of neuron ensembles to work in a coordinated fashion depends on the integrity of their synaptic connectivity. Physical proximity or arrangement likely reflects a given circuit’s integrity. Thus, neuron density, laminar positioning and the spatial arrangement of particular types of neurons are important characteristics of the cytoarchitecture within the cerebrum (Arnold et al., 1997). However, whereas neuron density and laminar positioning are relatively easy to quantify, aspects of dispersion and arrangement of neurons are less straightforward. Accordingly, almost no studies have so far been carried out to quantify the spatial arrangement of neurons, except of studies on schizophrenia. In this context, one study (Arnold et al., 1997) using a two-dimensional approach found a decreased dispersion index of layer III neurons in the human entorhinal cortex in schizophrenia, suggesting a less clustered arrangement of these neurons compared to controls. The authors have interpreted this finding as being compatible with a disturbance of neuronal migration during brain development. On the other hand, neuronal densities in the entorhinal cortex in schizophrenia seem to be unaffected (Arnold et al., 1995), or slightly increased (Krimer et al., 1997). Other studies have reported an increased neuronal density in certain regions of the cerebral cortex in schizophrenia (Selemon et al., 1995, 1998), although the total neuronal number and the overall neuronal density seems to be unaffected in the schizophrenic brain (Thune et al., 2001). It is currently unknown whether or not this increased neuronal density in certain regions of the cerebral cortex in schizophrenia is related to alterations in the spatial arrangement of the neurons. Interestingly, a subtle but significant increase in the frequency of larger NNDs between layer III neurons has been found in the cingulate cortex in schizophrenia (Benes and Bird, 1987) and there is some indication of reduced volume of the cingulate cortex in schizophrenia (Szeszko et al., 2000). Because there is substantial overlap in the timing of developmental events for the cingulate and entorhinal cortices (Kostovic and Krmpotic, 1976; Muller and O’Rahilly, 1990), abnormal genetic or environmental mechanisms that disturb the cytoarchitectural development of one area might be expected to cause similar disturbances in the other. Considering that prenatal X-irradiation — as well as many other environmental aspects — might be a risk factor for schizophrenia (Tsuang, 2000), application of the method presented here to the human brain seems to have considerable relevance for further elucidation of the etiopathogenesis of this disease.

In schizophrenia, increased neuronal density but lack of neuronal loss may result in reduced neuropil volume (Selemon et al., 1995; Selemon and Goldman-Rakic, 1999). Because a large component of the neuropil is comprised of the dendritic processes of cortical neurons and presynaptic inputs onto cortical neurons, these processes would be reduced in number or size (Selemon and Goldman-Rakic, 1999). Thus, the disturbances in cognitive function in schizophrenia may result from the atrophy of neuronal processes, but stops short of actual neuronal loss (Selemon et al., 1995). Recent studies of cell size in schizophrenia provide indirect support for this hypothesis as neurons, particularly large pyramidal neurons, were found to have smaller somal sizes in the schizophrenic cortex (Rajkowska et al., 1998; Pierri et al., 2001). Since somal size correlates with the extent and complexity of dendritic arborization, this reduction suggests that pyramidal cells undergo atrophic changes in schizophrenia that may impair intercellular communication (Selemon and Goldman-Rakic, 1999). It is quite possible that similar effects take place in the mouse brain following prenatal low-dose X-irradiation. Several studies on prenatal X-irradiation with doses higher than used in the present study have pointed to an impaired development of pyramidal cell dendrites (Donoso and Norton, 1982; Fukui et al., 1991; Ochiai et al., 1993). In this context, one study (Norton, 1979) is particularly relevant: pregnant rats irradiated with 125 cGy on day 15 of gestation (i.e. close to day 13 of gestation of the mouse) had telencephalic neurons developing synaptic connections on dendrites during maturation which appeared to be normal spines in Golgi-stained light microscope preparations. At 6 weeks of postnatal age, both control and irradiated rats had spiny dendritic processes on cortical pyramidal cells and Golgi type II neurons in the caudate nucleus. However, with 6-month-old rats, the irradiated animals had more neurons with beaded dendritic processes that lacked spines or had only a few spines. These neurons resembled the neonatal neurons and were likely to be degenerating (Norton, 1979). Interestingly, in our recent study on the same animals as investigated in the present study we found a significant reduction of ∼16% of the total number of hippocampal pyramidal cells, which was not observed in littermates of these mice on postnatal day 25 (Korr et al., 2001). This indicates that degeneration of certain pyramidal cells following prenatal low-dose X-irradiation occurs in the mouse brain. In addition molecular/morphologic analyses we found indirect evidence that this cell loss was most probably due to accumulated mitochondrial DNA damage leading to high levels of free radicals, following prenatal low-dose X-irradiation (Korr et al., 2001). Prenatal X-irradiation-induced accumulation of mitochondrial DNA damage and increased amounts of free radicals could also be inferred for the layer V pyramidal cells, but to a considerably lesser degree than for the hippocampal pyramidal cells (Korr et al., 2001). Most probably, this may account for the differences in the vulnerability of neocortical and hippocampal pyramidal cells to prenatal X-irradiation-induced cell death. It is not known whether similar processes may also be found in the schizophrenic brain. However, it is worth noting that there is some evidence for increased levels of free radicals in schizophrenia (Reddy and Yao, 1996; Yao et al., 2000, 2001).

In conclusion, the present data indicate that a more dispersed spatial arrangement of layer V pyramidal cells and a reduced reference space containing these cells, but no alteration in their numbers, result from low-dose prenatal X-irradiation in mice. Because abnormal neuronal migration may be a primary cause of this altered neuronal spatial arrangement, the present observations validate further prenatal X-irradiation as a laboratory animal model of neuronal migration disorders affecting the human brain. In addition, it appears that this model may be helpful to our understanding of the morphologic and biochemical alterations of the schizophrenic brain. Finally, the quantitative approach presented here permits the 3D analysis of...
the spatial arrangement of neurons without any kind of pretreatment, such as labeling neuronal precursors with BrdU, and without edge effect bias, is highly accurate and sensitive and can be used easily to study any pathologic disturbances of neuronal distribution in both animal models and the human brain.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oupjournals.org/cgi/content/full/12/9/954/DC1.

Notes
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Address correspondence to Christoph Schmitz, Department of Anatomy and Cell Biology, RWTH University of Aachen, Wendlingweg 2, D-52057 Aachen, Germany. Email: cschmitz@ukaachen.de.

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