Published in final edited form as:

J Comp Neurol. 2013 April 1; 521(5): 1040–1053. doi:10.1002/cne.23217.

Distinct abnormalities of the primate prefrontal cortex caused by ionizing radiation in early or midgestation

Lynn D. Selemon¹, Can Ceritoglu³, J.Tilak Ratnanather³, Lei Wang⁴, Michael P. Harms⁵, Kristina Aldridge⁶, Anita Begovic¹, John G. Csernansky⁴, Michael I. Miller³, and Pasko Rakic^{1,2}

¹Department of Neurobiology at Yale University, New Haven, CT

²Kavli Institute for Neuroscience at Yale University, New Haven, CT

³Center for Imaging Science and Institute for Computational Medicine, The Johns Hopkins University, Baltimore, MD

⁴Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine, Chicago, IL

⁵Department of Psychiatry, Washington University School of Medicine, St. Louis, MO

⁶Department of Pathology and Anatomical Sciences, University of Missouri School of Medicine, Columbia, MO 65212

Abstract

Prenatal exposure of the brain to environmental insult causes different neurological symptoms and behavioral outcomes depending on the time of exposure. To examine the cellular bases for these differences, we exposed Rhesus macaque fetuses to x-rays during early gestation (E30–E42), i.e., before the onset of corticogenesis, or in midgestation (E70–E81) when superficial cortical layers are generated. Animals were delivered at term (~E165), and the size and cellular composition of prefrontal association cortex (area 46) examined in adults, using magnetic resonance imaging (MRI) and stereologic analysis. Both early and midgestational radiation exposure diminished the surface area and volume of area 46. However, early exposure spared cortical thickness and did not alter laminar composition, and due to higher cell density, neuron number was within the normal range. In contrast, exposure to x-rays at midgestation reduced cortical thickness, mainly due to elimination of neurons destined for the superficial layers. A cell-sparse gap, observed within layer III, was not filled by the later generated neurons destined for layer II, indicating that there is no subsequent replacement of the lost neurons. The distinct areal and laminar pathology consequent to temporally segregated irradiation is consistent with basic postulates of the radial unit hypothesis of cortical development. In addition, we show that an environmental disturbance inflicted in early gestation can induce subtle cytoarchitectonic alterations without loss of neurons, such as those observed in schizophrenia, whereas midgestational exposure causes selective elimination of

Corresponding author: Lynn D. Selemon, Ph.D. Department of Neurobiology Yale University School of Medicine PO Box 208001 New Haven, CT 06520-8001 Phone: 508 540-5306 FAX: 203 785-5263 ldselemon@aol.com.

Conflict of Interest Statement The authors have no competing financial interests with this manuscript.

Author Contributions All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. LDS and PR designed the experiments, supervised prenatal irradiation procedures and wrote the manuscript. LDS and AB performed cytoarchitectonic delineations and stereologic analyses. CC, JTR, LW and MIM developed the registration method that allowed transfer of cortical boundaries from histology sections to MRI images, and CC and JTR conducted morphometric measures of area 46 in MRI volumes. MPH and LW assisted with MRI collection and MPH with statistical analysis of the data. KA performed the total cortical gray matter volume measurements. JGC assisted with the design of the histology-registration method and the MRI analyses.

neurons and cortical thinning as observed in some forms of mental retardation and fetal alcohol syndrome.

Keywords

magnetic resonance imaging; neurodevelopment; thalamocortical; schizophrenia; stereology

It is generally agreed that the same prenatal environmental factors cause remarkably different brain pathology depending on when the exposure occurs during gestation (Rakic, 1988a; 1988b). In the present study we have tested this hypothesis by irradiating macaque monkeys in early gestation, i.e., prior to the onset of corticogenesis, and in midgestation, i.e., during the peak of corticogenesis, and then examining the outcome during adulthood by *in vivo* magnetic resonance imaging (MRI), followed by postmortem stereologic analysis of cytoarchitectonic changes. The study was undertaken to determine whether the size and/or cellular composition of the dorsolateral prefrontal cortex (dlPFC), area 46 of Walker (1940), is altered differentially by prenatal irradiation during early versus midgestation.

Our first objective was to compare the pathology in area 46 consequent to early and midgestational irradiation. The radial unit hypothesis posits that genetic and environmental changes should affect formation of the cortex differentially and in a predictable manner if exerted at early or late developmental stages (Rakic, 1988a). It is well established that generation of the cerebral cortex in both human and non-human primates proceeds in a two-step process in embryonic development: in early development (i.e., before E40 in macaques and E42 in humans), symmetrical division of cortical progenitor cells gives rise to additional cortical progenitor units thereby expanding the cortical protomap, while later (i.e., after E40/E42), asymmetric division of progenitor cells produces neural precursor cells and generates columns of cortical neurons spanning the thickness of the cortex (Sidman and Rakic, 1973; Rakic, 1988a, 1995; Bystron et al. 2008). Furthermore, the temporal staggering of corticogenesis has functional implications as early generated neurons develop mainly cortico-subcortical projections whereas later generated neurons form mostly cortico-cortical and local synaptic connections (Jones, 1986). Accordingly, we hypothesized that irradiation before corticogenesis would diminish the expanse of area 46 across the cortical mantle, whereas irradiation during corticogenesis would result in a thinner cortex due to generation of fewer neurons in each cortical column. In light of the deleterious effects of early gestational irradiation on thalamic number, particularly in the medial dorsal nucleus (MD) (Selemon et al., 2009), we also considered the possibility that partial thalamic deafferentation might diminish the expanse of area 46, as previous studies in humans and macaques have established that thalamic input plays a major role in determining cortical specification (Eidelberg and Galaburda, 1982; Rakic, 1988a; Rakic et al., 1991; Suner and Rakic, 1996).

The second goal of our study was to examine whether disruption of neurogenesis in either early or midgestation would produce pathology consistent with that observed in neurodevelopmental disorders. For this reason, we focused on the dlPFC whose dysfunction is a prominent feature of several neuropsychiatric disorders including schizophrenia (Goldman-Rakic, 1991, 1994; Barch, 2005). Walker's area 46 of the dlPFC mediates spatial mnemonic function in the non-human primate (Goldman-Rakic, 1987), and neuroimaging data have shown that the equivalent area in human, Brodmann area 46, is critical for performance on executive tasks like the Wisconsin Card Sort and N-Back that rely on working memory capacity and are disrupted in schizophrenia (Weinberger et al., 1986; Carter et al., 1998). Interestingly, a recent MRI analysis examined separately the two components of volume, i.e. area and thickness, for regions of the frontal cortex and found

selective reduction of cortical area with sparing of thickness in schizophrenia subjects (Gutierrez-Galve et al., 2010). In contrast, individuals with some forms of mental retardation, as well as fetal exposure to alcohol, nicotine, opiates and other substances which lead to profound cognitive impairment, exhibit marked cortical thinning (Walhovd et al., 2007; Toro et al., 2008; Lotfipour et al., 2009; Zhang et al., 2011; Zhou et al., 2011). In light of the fact that the neurogenerative mechanisms that determine cortical expanse and cortical thickness are temporally separate developmental events controlled by distinct genetic influences (Rakic, 1988a, 1995; Bystron et al., 2008; Joyner et al., 2009; Pannizzon et al., 2009), these findings suggest that selective reduction of surface area, as observed in schizophrenia, may be due to disruption of early gestational brain development and that cortical thinning, as observed in a number of human psychiatric conditions, may be associated with disturbances impacting developmental processes in midgestation.

In the present study we have obtained novel measures that combine *in vivo* neuroimaging and postmortem histologic analyses of the same brains (Certigolu et al., 2010). This new strategy enabled us to precisely transfer the cytoarchitectonically defined borders of area 46 from the histology sections to the MRI volumes so that measures of surface area, volume and cortical thickness would not be confounded by shrinkage or distortion due to postmortem fixation, dehydration and processing. Stereologic analyses were then performed on the histology sections to assess the cellular bases of these structural alterations.

Materials and Methods

Subjects

Twenty-two Rhesus macaques were the subjects of this study: eight early gestationally irradiated animals (EGAs), three midgestationally irradiated animals (MGAs), and eleven controls (CONs), consisting of seven non-irradiated controls (nCONs) and four sham-irradiated controls (shCONs) (Table 1). The brains of all irradiated monkeys and eight controls (4 nCONs; 4 shCONs) were structurally imaged *in vivo*. An additional three nCONs were included only in the postmortem stereologic analyses. The monkeys represent two cohorts of animals (Table 1) that differ with respect to mode of birth, maternal care, housing, age at sacrifice, scanning, and stereologic analysis as will be described. Data from the two cohorts were combined to increase the power of the study. All procedures involving non-human primate subjects were performed in accordance with guidelines established by the Yale Institutional Animal Care and Use Committee.

Cohort generation: Fetal irradiation of monkeys was originally undertaken to compare the effects of early and midgestational irradiation on the visual system. Because the irradiation procedure was in an exploratory phase, varying doses of radiation were tested in different animals (Algan and Rakic, 1997). Most of these monkeys were sacrificed at young ages. Cohort 1 consists of the three EGAs and three MGAs from this original cohort that were allowed to mature to adulthood, along with non-irradiated CONs. Exposure to radiation was higher for the adult surviving MGAs than for the EGAs (Table 1). Cohort 2 was generated nearly a decade later to more fully examine the effects of radiation in the early gestational period. Guided by the findings from Algan and Rakic (1997), lower doses of radiation (comparable to that of EGAs from Cohort 1) were selected because these had proved to be effective in killing targeted cell populations with minimal possibility of non-specific effects, i.e. damage to cell populations that were not undergoing final mitosis at the time of exposure. Cohort 2 also includes sham-irradiated CONs.

Cohort 1: Three EGAs, three MGAs, and six CONs (including three CONs included only for stereologic analysis) were born via Caesarian section at full term (~E165). After birth, prenatally irradiated monkeys were raised in the Yale primate nursery for six months before

transfer to the adult primate colony. CONs were normal monkeys that had never been exposed to irradiation (nCONs) and were purchased from outside vendors. All monkeys were housed individually in the adult Yale colony. As adults (5–10 yrs old), the monkeys underwent whole brain imaging and were subsequently (6–12 yrs old) sacrificed for anatomical studies (Table 1). Three additional nCONs that had not been imaged were sacrificed as adults (4.8–14 yrs old).

Cohort 2: Four EGAs, three shCONs, and one nCON monkeys were born naturally at term (E165) and stayed with their mothers until weaning at 6 months of age. Six were then pair-housed and two (nCON-Vi and EGA-Sa) were singly housed in the adult colony. ShCON-La was delivered by Caesarian section when the pregnancy went beyond the expected date of delivery; attempts to reunite her with her mother were unsuccessful. EGA-Aj was delivered naturally but rejected by his mother. These two monkeys were raised in the primate nursery for six months and then pair-housed in the adult colony. All ten monkeys were scanned as adults and sacrificed shortly thereafter (Table 1).

Prenatal irradiation exposure

The irradiation protocol has been described in detail previously (Algan and Rakic, 1997). Pregnant monkeys were sedated with ketamine (5–10 mg/kg), administered in conjunction with atropine (0.02 mg/kg). Ultrasound was used to locate the head of the fetus and to estimate the depth of the head from the abdominal surface. A 250 kV, 15 mA Stabilipan x-ray tube delivered a beam of irradiation (area 5×5 cm) to the head of the fetus. Head depth was factored into the determination of the duration of the exposure necessary to achieve the target dose of irradiation (Table 1).

Magnetic resonance imaging (MRI)

Cohort 1: For 8 of the 9 monkeys, high resolution MRI scans were acquired on a 1.5 Tesla LX GE Signa scanner using a T1-weighted, 3D spoiled gradient recalled acquisition at steady state (SPGR) sequence with voxel resolution of 0.625×0.625 mm and slice thickness of 0.7 mm. Due to an institutional change in scanner availability, nCON-Sa (Cohort 1) and all monkeys in Cohort 2 were scanned as follows. Cohort 2: Scans were acquired on a 3T Trio scanner using a 3D MPRAGE sequence with voxel size of 0.625×0.625 mm for all Cohort 2 monkeys except shCON-Ma, shCON-Vi, and EGA-Tr in which voxel size was 0.586×0.586 mm. Slice thickness for all scans was 0.6 mm.

Perfusion and histological processing

Monkeys were sedated with ketamine (5–10 mg/kg IM) and deeply anesthetized with sodium pentobarbital (100 mg/kg IV) then perfused intracardially with 0.1 M phosphate buffered saline followed by a mixed aldehyde fixative (Cohort 1: 4% paraformaldehyde + 0.2% glutaraldehyde; Cohort 2: 4% paraformaldehyde + 0.1% glutaraldehyde + 15% saturated picric acid) in 0.1 M phosphate buffer (pH 7.4, 4° C). The left frontal lobe was embedded in celloidin and serially sectioned coronally at 40 μ m. Sections were Nissl-stained for cytoarchitectonic delineation of area 46 and stereologic analysis.

Cytoarchitectonic delineation of area 46

Area 46 occupies the sulcal walls and depths of the principal sulcus (Fig. 1A). Area 46, according to Walker (1940), is characterized by a thick granular layer IV, marked radial striations, a wide layer I and indistinct boundaries between layers V and VI (Fig 1B). Supragranular layers II and III are wider than infragranular layers V and VI. Area 46 extends nearly to the frontal pole anteriorly where it is bounded by area 10, a thinner cortex with medium-sized pyramidal cells in layer V. Along the length of the principal sulcus, area

46 extends onto the convexity of the superior frontal gyrus and is bordered by area 9 anteriorly and area 8 posteriorly (Fig. 1A,D). Area 9 can be distinguished from area 46 because it lacks the radial striations of area 46, exhibits a generally less organized structure, and has a relatively thinner layer IV (Fig. 1D). Area 8 is characterized by a much thinner layer IV and the presence of large pyramidal cells in layer V. Where area 46 extends onto the inferior frontal convexity, it is bounded by area 12 anteriorly and area 45 posteriorly (Fig. 1A, C). Areas 12 and 45 also lack the pronounced striations of area 46 and have a less pronounced layer IV (Fig. 1C). In addition, area 45 is marked by the presence of large pyramidal neurons in layers III and V.

Area 46 boundaries with adjacent cortical areas were defined by a single author (LDS) for Cohort 1 and by two authors (LDS and AB) for Cohort 2 in order to assess reliability of border placement. All MRI computations of volume, surface area, and cortical thickness were performed twice using the two border delineations; means of the two computed values were used for each monkey. The average percent differences in area 46 metrics due to interrater differences in border placement were small: volume (8.5%), surface area (2.8%), cortical thickness (3.8%), and two-tailed Pearson's interrater correlations ($N=19$) were all highly significant (volume: 0.931, $p < 0.001$; surface area: 0.987, $p < 0.001$; cortical thickness: 0.883, $p = 0.001$). Laminar boundaries in all brains were defined by LDS.

Transfer of the cytoarchitectonic and laminar boundaries of area 46 from histologic sections to MRI volumes

A multistep process based on large deformation diffeomorphic mapping (LDDMM) (Joshi and Miller, 2000; Beg et al., 2005) was used to transfer area 46 boundaries to the MRI scans as described in detail in Ceritoglu et al. (2010). Gray matter and white matter compartments were manually segmented in a series of 1 in 20 (1 in 10 for Cohort 2) coronally cut, Nissl stained sections spanning area 46. Consecutive sections were aligned using rigid registration and then stacked to form a 3D volume in which the gray matter and white matter regions were labeled with different intensity values (128 for GM and 255 for WM). In the MRI image, the prefrontal cortex gray matter and white matter compartments were also manually segmented as described previously (Selemon et al. 2005) and labeled with different intensity values (128 for GM and 255 for WM). To align the 3D histologic volume with the 3D MRI volume, an affine transformation between these two images was computed using the Automated Image Registration (AIR) program which minimizes the least square error cost function. Finally, a non-linear LDDMM registration method was used to complete registration between the histological and MRI volumes. Area 46 boundaries and laminar borders, defined in the histological sections, were transferred onto the MRI volume via LDDMM driven by the intensity changes at the GM/WM border in the segmented images.

Measurement of area 46 surface area, volume and cortical thickness in MRI volumes

In MRI volumes, Labeled Cortical Depth Mapping (LCDM) was used to compute surface area and volume as described in Miller et al. (2000; 2003). With this method every segmented GM voxel is labeled by its distance to the GM/WM surface giving rise to a histogram from which volume and thickness can be derived via summation and calculation of the 95th percentile, respectively. Surface area is computed by simple triangulation. The same principles were applied for measurement of volume and thickness in the three defined cortical layers. Volumes were not corrected for intracranial volume or total cerebral volume since these corrections limit the power to detect group differences in developmental studies in which all individuals of one group are expected to have smaller brain size overall (Arndt et al., 1991; Mathalon et al., 1993).

Total cortical gray matter (GM) volume

Total cortical GM volume was measured in Cohort 2 according to the published protocol used for analysis of cortical GM volume in Cohort 1 animals (Selemon et al., 2005). Values for frontal GM volume and non-frontal GM volume, as reported in Selemon et al. (2005), were summed to yield total cerebral cortical GM volume for Cohort 1. Percent total GM volume was computed as area 46 volume/total cerebral cortical GM volume multiplied by 100.

Stereologic analyses

Area 46 total cell number—All stereologic analyses of area 46 were conducted on a Zeiss Axioskop microscope fitted with an integrated, computer-guided Lucivid system and Stereo Investigator (ver. 5.05) software (MicroBrightfield, Williston, VT). Slides were coded so that analyses were performed blind to experimental status by a single observer (LDS). The total number of neurons and glia in area 46 was estimated using the optical disector method (Gundersen, 1986), and systematic, random sampling was achieved by applying the fractionator method of grid-based sampling (Gundersen et al., 1988). Every 20th section was analyzed, and a sampling grid of 1000 $\mu\text{m} \times 700 \mu\text{m}$ was used. A contour of the area of interest (area 46) was drawn at low power (90 $\times$; 2.5 $\times$ air objective, numerical aperture = 0.075), and cells were counted under high power (3600 $\times$; 100 $\times$ oil immersion objective; numerical aperture = 1.30). Contour boundaries for Cohort 2 were based on the mean distance between boundaries determined by AB and LDS.

An optical disector of 60 (l) $\times$ 45 (w) $\times$ 20 (h) μm (60 $\times$ 40 $\times$ 20 for Cohort 2) was chosen to maximize the size of the box but still allow for 2 μm guard zones at the top and bottom of the section. Neuronal nuclei and glial nuclei inside the box were counted. Section thickness was measured at each optical disector, and total number was estimated based on number weighted section thickness. For two brains (identified later as EGA-Bu and EGA-Lo), mean measured section thickness was greater than 40 μm . Based on shrinkage of other brains in Cohort 2, it was determined that these two brains were inadvertently sectioned at 60 μm . These brains were recounted using 60 μm as the cut section thickness and block advance. A coefficient of error (CE) (Gundersen et al., 1999) of 0.02 was calculated for neuron and glia counts for all brains, with the exception of MGA-Ob who had a CE of 0.01 for both neuron and glia counts.

Area 46 cell density—Cell density was derived from total neuron or glial cell number divided by volume of area 46 as measured in MRI volumes.

MD total neuron number—Neuron number in MD for Cohort 2 and nCON-Sa of Cohort 1 was estimated according to the previously published protocol used in the analysis of Cohort 1 animals (Selemon et al., 2009). All MD cell counts were performed by a single investigator (AB), who also performed previously published MD cell counts for Cohort 1. CEs were ranged from 0.03–0.04 for all monkeys.

Statistics

Statistics were performed using SPSS (ver. 19.0). Normality of the data was established with a Kolmogorov-Smirnov test. Regression and analysis of variance (ANOVA) functions of the General Linear Model were used to test for statistical significance. Regression was used to assess the influence of age at sacrifice on neuron number and glia number and to examine the effect of age at MRI on volume, surface area, cortical thickness, and neuronal and glial density. Differences in group means were tested using a univariate ANOVA with post-hoc pairwise comparisons using a Bonferroni correction for multiple comparisons. ANOVAs were also performed covarying for cohort. Regression analysis was performed to examine

the relationship between MD neuron number and area 46 volume, surface area and cortical thickness. All statistical tests were considered significant at the $p < 0.05$ level. Data are expressed as mean + standard deviation (SD).

Preparation of photomicrographs

Selected sections were scanned on an Aperio Scanscope or photographed as viewed on a Zeiss Axioskop2 plus microscope with a Nikon CoolPix 990 camera. The digital images were sized and adjusted for brightness and contrast in Adobe Photoshop.

Results

Influence of age and cohort

Neuronal density showed a trend correlation ($R^2 = 0.124$; $p = 0.077$) with age at MRI in the combined cohorts. When the correlations were performed separately by cohort, the correlation was significant in Cohort 1 ($R^2 = 0.457$, $p = 0.027$). No other regressions with age at sacrifice or age at MRI were significant. Because of the effect of age at MRI on neuronal density in Cohort 1 and the age disparity in Cohort 1 (Table 2), the two cohorts were analyzed separately for neuronal density with age at MRI included as a covariate in the ANOVA.

Only area 46 surface area showed a significant cohort effect (0.05). However, the group effect remained significant when covarying for cohort ($F(2,15) = 6.456$; $p = 0.011$). Means for both CONs and EGAs were lower in Cohort 1 compared to Cohort 2.

Area 46 volume, surface area, and cortical thickness in MRI volumes

Both irradiated groups exhibited reductions in overall volume and surface expanse of area 46. Volume was reduced by 20% and surface area by 19% for EGAs, whereas greater reductions of 43% volume and 40% surface area were observed in MGAs (Table 2; Fig. 2A,B). Area 46 volume in MGAs tended to be smaller than that of EGAs; surface area did not differ between the irradiated groups (Table 2, Fig. 2A,B). Cortical thickness was reduced selectively in MGAs by 13% compared to CONs and by 14% compared to EGAs (Table 2, Fig. 2C).

Laminar volume and thickness in MRI volumes

In EGAs all three layers exhibited volume reductions although the decrease in layer I reached only a trend level of significance (Table 2, Fig. 3A). Layer II–IV volume was reduced by 23%, and layer V–VI volume was decreased by 17% compared to CONs. In MGAs, laminar volume reduction was most pronounced in supragranular/granular layer II–IV with MGA volume reduced by 62% compared to CONs (Table 2, Fig. 3A). Layer I volume was reduced by 43% in MGAs relative to CONs whereas layer V–VI volume was not significantly different in MGAs and CONs (Table 2, Fig. 3A). Layer II–IV volume in MGAs was only half as large as that of EGAs, and layer I tended to be smaller in MGAs than in EGAs (Table 2, Fig. 3A).

Laminar thickness was specifically and selectively reduced only in layer II–IV of the MGA group (Table 2, Fig. 3B). Layer II–IV was 28% thinner in MGAs than in CONs and 24% thinner than that of EGAs (Table 2, Fig. 3B). In addition to diminished volume and thickness of supragranular/granular layers of the cortex, all three MGAs also exhibited a cell-sparse band in the supragranular layers and an altered proportion of supra- to infragranular layers compared to controls (Fig. 4).

Area 46 total cell number and cell density

MGA neuron number tended to be lower than that of CONs (Table 2, Fig. 5A). No other pairwise comparisons approached significance. Total glia number did not differ among groups nor did neuron to glia ratio (Table 2, Fig. 5B). Neuronal densities, i.e., as calculated by dividing total cell number by volume of area 46, did not differ among the three groups in Cohort 1 (Table 2). In Cohort 2, in which the groups were well matched for age, neuronal density was elevated by 21% in EGAs compared to CONs (Table 2, Fig. 6). Glia density was not altered in either irradiated group compared to CONs (Table 2). It should be noted that cell number and cell density in the CONs was comparable to previous reports based on sulcal parcellation of the dlPFC (Dombrowski et al., 2001; Christensen et al., 2007).

Percent of Area 46 Volume compared to Total Cortical GM Volume

Mean percent of area 46 volume relative to total GM volume did not differ from CONs in EGAs, and there was only a trend reduction in MGAs compared to CONs (Table 2). Notably, total GM volume was significantly decreased in both irradiated groups, by 17% in EGAs and by 31% in MGAs, compared to CONs (Table 2). EGA and MGA total GM volumes did not differ from each other. In addition, group differences in area 46 volumes were not found when total GM volume was included as a covariate in the ANCOVA ($F(2,13)=0.973$, $p=0.404$).

Correlation with MD total neuron number

Regression analysis showed a significant correlation between MD neuron number and area 46 volume ($R^2=0.268$, $p=0.04$), a trend correlation between MD neuron number and area 46 surface area ($R^2=0.214$, $p=0.071$), and no relationship between MD neuron number and area 46 cortical thickness ($R^2=0.021$, $p=0.593$). CON and EGA groups were combined for the analyses. MD cell counts were not available for MGAs. MD neuron number showed only a trend reduction in EGAs compared to CONs in the present study (Table 2).

Discussion

The main effect of ionizing radiation is non-selective killing of dividing cells (Sinclair and Morton, 1966). Cortical development is a highly dynamic process in which the fate of newly generated cells changes with embryonic age; therefore, radiation exposure and the ensuing disruption of cellular genesis results in different pathologies in the adult brain depending on the time of exposure. Fetal exposures in the present study targeted two discrete developmental epochs, the first during production of neural stem cells but prior to the onset of corticogenesis and the second during the peak of corticogenesis when production of neurons destined to form supragranular layers of the cortex are generated. Our comparative analysis of the prefrontal cortex in adult animals revealed four main findings 1) Killing neural stem cells at the early stages decreases both the surface and volume of area 46, but surprisingly preserves total neuron number, as neurons are more densely packed, and normal laminar architecture is retained; 2) killing of cortical progenitors during midgestation results in selective elimination of neurons destined for the supragranular cortical layers resulting in distorted laminar proportions; 3) eliminated neurons are not replaced by the subsequently generated populations of cells that bypass depleted layers to settle in a more superficial strata; 4) cortical abnormalities consequent to irradiation during different prenatal ages are reminiscent of pathologies observed in different human psychiatric disorders.

Adult pathology following early versus midgestational x-irradiation

The distinct patterns of cortical abnormalities associated with early versus midgestational x-irradiation are generally consistent with the two-phase process of corticogenesis and support

the main postulates of the radial unit hypothesis of cortical development and evolution (Rakic, 1988a, 1995, 2009; Bystron et al. 2008). For example, cortical thickness was not abnormal in EGAs but was thinner following midgestational irradiation. Furthermore, the pathology in MGAs was laminar-specific since, as expected, both cortical thinning and loss of volume in MGAs were most pronounced in layer II–IV, the layer that is generated at the time of irradiation. Thus, the selective pathologic involvement of supragranular layers in midgestationally irradiated monkeys illustrates the manner in which circumscribed populations of neurons can be targeted by prenatal perturbation due to the temporal segregation of neurogenesis across cortical layers (Rakic, 1988a).

In contrast, the reduction in surface area in the MGAs was not predicted by the radial unit hypothesis since the number of embryonic radial columns is complete by midgestation (Rakic, 1988a, 1995). However, this apparent contradiction can be explained by multiple effects of x-irradiation and secondary consequences of eliminated and damaged neurons. For example, contraction of the cortical mantle in MGAs may occur after completion of development due to the elimination of layer III neurons and their axons that form massive corticocortical projections and are essential for normal growth and maturation of the neocortex. Another factor that may have contributed to cortical shrinkage is that the irradiation dose for MGAs was higher (300–600 cGy) than for EGAs (150–200 cGy). A prior study of fetal irradiation effects in the visual system suggested that doses >200cGy produce ventricular enlargement in conjunction with decreased white matter volume (Algan and Rakic, 1997). Given the disparity in radiation exposure in the two groups, we cannot entirely discount the possibility that the more severe reduction of volume and surface area in MGAs compared to EGAs is due to differences in dosage. However, high doses of radiation in midgestation did not produce any measurable pathology in the thalamus (Schindler et al., 2002; Selemon et al., 2009) and resulted in laminar-specific pathology in the prefrontal cortex in the present study, suggesting that the effects of radiation exposure are region-specific and even layer-specific in this dose range. In fact, thalamic neuron number was normal for MGA-Ob (published in Selemon et al., 2009), the only MGA in which thalamus was available for study.

Developmental mechanisms

One possible explanation for the reduction of area 46 volume and surface area following early gestational irradiation is that irradiation prior to the onset of corticogenesis eliminated pluripotential neural stem cells which divide symmetrically, thereby reducing the number of proliferative units in the ventricular zone with consequent reduction of cortical surface (Rakic, 1988a, 1995). Another possibility is that the cortical expanse of area 46 in EGAs was reduced as an indirect result of elimination of thalamocortical projection neurons since thalamic neurons also are generated during the early gestational exposure period (Rakic, 1977). Recent analysis of the thalamus in Cohort 1 animals uncovered pronounced reduction of neuron number in MD (Selemon et al. 2009), the primary source of thalamic afferent input for area 46 (Goldman-Rakic and Porrino 1985; Giguere and Goldman-Rakic 1988). In the present dataset, we found a significant correlation between MD total cell number and area 46 volume and a trend level correlation with area 46 surface area. Certainly, these findings do not establish causality: that is, we cannot say whether MD number determines area 46 size, vice versa, or whether the correlation simply represents a coincidence between timing of generation of neurons in MD and cortical progenitor populations for area 46. Nonetheless, our results are consistent with prior studies in humans and primates that have established a proportionate relationship between thalamic afferent populations and targeted cortical area size (Eidelberg and Galaburda 1982; Rakic 1988a; Rakic et al. 1991; Suner and Rakic 1996).

In either case, i.e., whether surface area is reduced due to killing of cortical neural stem cells or due to reduction of thalamocortical afferent input, the expected outcome would be reduced neuron number in area 46. Neuron number was not reduced in the EGA group, although variability was quite high, and six of the eight animals had neuron numbers below the CON mean. It is therefore possible that a larger sample of brains would have enabled detection of reduced neuron number.

Layer III neurons are not replenished by subsequent neurogenesis

We observed a cell-sparse gap in the supragranular layers of area 46 in adult animals exposed to irradiation in midgestation that corresponds to the location that would have been occupied by radiation-killed cells. The fact that this gap persisted into adulthood indicates that later generated neurons have migrated to more superficial locations in the cortical mantle bypassing this unpopulated stratum, continuing to their predestined cortical position, and leaving a gap behind. This is the expected outcome since basic neuronal phenotypes are determined during the last cell division in the ventricular proliferative zone (Caviness and Rakic, 1978; McConnell, 1988; Rakic et al., 2009). The presence of a cell-sparse gap in fully adult animals indicates failure not only of normal developmental mechanisms but also of subsequent regenerative capacity of neural stem cells or astroblasts to compensate for neuronal loss in the cortex. As such these findings further underscore the difficulties that may be encountered by efforts to transplant and/or induce neural stems in the adult human brain to repair brain damage (Breunig et al, 2011).

Advantages of the combined histology/MRI approach

We used histologic analysis of cytoarchitectonic boundaries to “inform” the MRI analyses of volume, surface area and cortical thickness of area 46. In combining these two techniques, we drew on the strengths of both methodologies, i.e., the precise delineation of cytoarchitectonically distinct and therefore more functionally homogenous units of brain structure, as discernible on postmortem histology sections, and the visualization of *in vivo* brain structure, undistorted by postmortem fixation and processing, on MRI scans. Since cytoarchitectonic studies of human prefrontal cortex indicate substantial variability in the location of cortical areas across subjects and little concordance with sulcal landmarks (Rajkowska and Goldman-Rakic 1995), the application of cytoarchitectonic borders to define regions of interest for *in vivo* structural imaging analyses could provide uniquely valuable information to improve the reliability and validity of comparisons of human populations. In the present study, further delineation of cortical layers within area 46 enabled us to pinpoint the pathology in MGAs to supragranular/granular layers. Cortical layers by definition are cytoarchitecturally distinct subunits of the cortex and differ functionally due to diverse connectivity with other brain regions (Jones 1986). Thus, the approach that we used in this study, i.e. histologic delineation of laminar boundaries to define regions of interest for the MRI analyses, could significantly enhance our understanding of the neuropathology of psychiatric illness if adapted for study of the human cortex.

Relevance to neuropsychiatric disease

Early gestational irradiation in the non-human primate resulted in adult cortical pathology that resembles the cortical pathology of schizophrenia in several respects. The surface expanse of area 46 in early irradiated animals was diminished while cortical thickness did not differ from that of controls, as has been found in subjects with schizophrenia (Guttlerez-Galve et al., 2010) albeit not universally (e.g., Harms et al., 2010). Neuronal density was higher in EGAs versus CONs in the absence of any change in total neuron number, similar to what has been described in postmortem brains from schizophrenia patients (Pakkenberg, 1993; Selemon et al., 1995; 1998; 2003), although there was considerable variability in total

neuron number in the EGA group as discussed above. Parallels between pathology following early gestation irradiation and pathology in schizophrenia lend support to a growing body of literature that implicates the early prenatal period as a critical window of vulnerability for schizophrenia (Susser and Lin, 1992; Hollister et al., 1996; Brown et al., 2000; Insel et al., 2008). It should be noted, however, that we did not find evidence for selectivity of pathology to the dlPFC, as has been described in schizophrenia subjects (Sullivan et al., 1998; Gur et al., 2000; Convit et al., 2001; Lopez-Garcia et al., 2006; Harms et al., 2010). Proportionate volume of area 46 to total gray matter volume in EGAs was not different from that of CONs. It is likely that progenitor cells destined to form the protomap for the entire cortical mantle were depleted by irradiation exposure resulting in widespread area and volume reduction. Global pathology is further supported by the presence of these same irradiation induced changes, i.e. reduction of surface area, volume, and increased neuronal density, in the visual cortex of early gestationally irradiated monkeys (Algan and Rakic, 1997). Interestingly, some evidence points to a more global pathology in schizophrenia, including cytoarchitectonic changes in the visual cortex of schizophrenia patients (Selemon et al., 1995; Dorph-Petersen et al., 2007; Sun et al., 2009).

The pathology observed in MGAs also bears some similarities to human neurodevelopmental pathology. In particular, cortical thinning has been observed in certain forms of mental retardation and in subjects who were exposed to substance abuse *in utero*, including alcohol, cigarette smoking, and opiates (Walhovd et al., 2007; Toro et al., 2008; Lotfipour et al., 2009; Zhang et al., 2011; Zhou et al., 2011). Because these studies are MRI-based, they cannot address the underlying cellular correlates of cortical thinning and therefore it is not possible to draw additional parallels to the laminar and cellular abnormalities observed in MGAs. Of course, substance abuse generally occurs throughout the prenatal period. Therefore it is possible that cortical surface area reduction is also associated with maternal substance abuse but has not as yet been reported since this parameter is less often analyzed in human MRI studies. Alternatively, exposure to alcohol, opiates and nicotine may selectively affect molecular pathways that are operative in the second phase of cortical development thereby impacting only cortical thickness. Recent studies have established that the two phases of development are controlled by different genetic influences and presumably separate molecular mechanisms (Pannizzon et al., 2009).

Studies of human populations suggest that many, diverse prenatal insults can adversely affect brain development. For example, maternal infection, maternal malnutrition, and rhesus (Rh) incompatibility all increase the risk for schizophrenia (Susser and Lin, 1992; Hollister et al., 1996; Brown et al., 2000; Insel et al., 2008), and maternal abuse of a wide range of drugs can lead to learning disabilities and cognitive impairment (Walhovd et al., 2007; Toro et al., 2008; Lotfipour et al., 2009). In the context of the present findings, the results of those studies might be interpreted to indicate that it is the timing rather than the nature of the insult that determines the pathologic outcome. That is, diverse forms of prenatal perturbation of the fetal environment may converge to produce similar disruptions in development. Recently, maternal inflammation has been shown to reduce proliferation in the ventricular zone (Stolp et al., 2011; 2012), much as radiation exposure in the present study appears to have decreased the number of cortical neural stem cells. Furthermore, genetic factors that alter development may mimic or act in synergy with environmental factors to derail the same neurodevelopmental process. As an example, recent studies have identified a role for disrupted in schizophrenia -1 (DISC1) in neurogenesis (Mao et al., 2009), suggesting that abnormal expression of this gene could impact neurodevelopment in a manner that is similar to the effects of radiation exposure on the prenatal brain.

Acknowledgments

We would like to recognize Dr. Oguz Algan for experimental treatment of the irradiated monkeys in Cohort 1 and also thank Heidi Voegeli, Heather Findlay, Marianne Horn, and Gary Leydon for excellent technical assistance.

This work was supported by the National Institutes of Health grants (RO1-MH 59329 to LDS, P50-MH44866 to Patricia S. Goldman-Rakic, Ph.D., P50-MH 71616 to JGC, P41-RR15241 to JTR and MIM, NY-014841 and EY-02593 to PR.

Literature Cited

- Algan O, Rakic P. Radiation-induced, lamina-specific deletions of neurons in the primate visual cortex. *J Comp Neurol*. 1997; 38:355–352.
- Arndt S, Cohen G, Alliger RJ, Swayze VW 2nd, Andreasen NC. Problems with ratio and proportion measures of imaged cerebral structures. *Psychiatry Res*. 1991; 40:79–89. [PubMed: 1946842]
- Barch DM. The cognitive neuroscience of schizophrenia. *Ann Rev Clin Psychol*. 2005; 1:321–353. [PubMed: 17716091]
- Beg MF, Miller MI, Troune A, Younes L. Computing large deformation metric mapping via geodesic flows of diffeomorphisms. *Internatl J Computer Vision*. 2005; 61:139–157.
- Breunig JJ, Haydar TF, Rakic P. Neural stem cells: historical perspective and future prospects. *Neuron*. 2011; 70:614–625. [PubMed: 21609820]
- Brown AS, Schaefer CA, Wyatt RJ, Goetz R, Begg MD, Gorman JM, Susser ES. Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. *Schizophr Bull*. 2000; 26:287–295. [PubMed: 10885631]
- Bystron I, Blakemore C, Rakic P. Development of the human cerebral cortex: Boulder Committee revisited. *Nat Rev Neurosci*. 2008; 9:110–122. [PubMed: 18209730]
- Carter CS, Perlstein W, Ganguli R, Brar J, Mintun M, Cohen JD. Functional hypofrontality and working memory dysfunction in schizophrenia. *Am J Psychiatry*. 1998; 155:1285–1287. [PubMed: 9734557]
- Caviness VS Jr, Rakic P. Mechanisms of cortical development: A view from mutations in mice. *Ann Rev Neurosci*. 1978; 1:297–326. [PubMed: 386903]
- Ceritoglu C, Wang L, Selemon LD, Csernansky JG, Miller MI, Ratnanather JT. Large deformation diffeomorphic metric mapping registration of reconstructed 3D histological section images and *in vivo* MR images. *Front Hum Neurosci*. 2010; 4:43. [PubMed: 20577633]
- Christensen JR, Larsen KB, Lisanby SH, Scalia J, Arango V, Dwork AJ, Pakkenberg B. Neocortical and hippocampal neuron and glial cell numbers in the rhesus monkey. *Anat Rec*. 2007; 290:330–340.
- Convit A, Wolf OT, deLeon MJ, Patalinjug M, Kandil E, Caraos C, Scherer A, Saing Louis LA, Cancro R. Volumetric analysis of the pre-frontal regions: findings in aging and schizophrenia. *Psychiatry Res*. 2001; 107:61–73. [PubMed: 11530273]
- Dombrowski SM, Hilgetag CC, Barbas H. Quantitative architecture distinguishes prefrontal cortical systems in the rhesus monkey. *Cereb Cortex*. 2001; 11:975–988. [PubMed: 11549620]
- Dorph-Petersen KA, Pierri JN, Wu Q, Sampson AR, Lewis DA. Primary visual cortex volume and total neuron number are reduced in schizophrenia. *J Comp Neurol*. 2007; 501:290–301. [PubMed: 17226750]
- Eidelberg D, Galaburda AM. Symmetry and asymmetry in the human posterior thalamus. I. Cytoarchitectonic analysis in normal persons. *Arch Neurol*. 1982; 39:325–332. [PubMed: 7046701]
- Giguere M, Goldman-Rakic PS. Mediodorsal nucleus: areal, laminar, and tangential distribution of afferents and efferents in the frontal lobe of rhesus monkeys. *J Comp Neurol*. 1988; 277:195–213. [PubMed: 2466057]
- Goldman-Rakic, PS. Circuitry of the prefrontal cortex and the regulation of behavior by representational knowledge. In: Plum, F.; Mountcastle, V., editors. *Handbook of Physiology*. Vol. vol. 5. American Physiological Society; Bethesda (MD): 1987. p. 373–417.

- Goldman-Rakic, PS. Prefrontal cortical dysfunction in schizophrenia: The relevance of working memory. In: Carroll, BJ.; Barrett, JE., editors. *Psychopathology and the Brain*. Raven Press; New York (NY): 1991. p. 1-23.
- Goldman-Rakic PS. Working memory dysfunction in schizophrenia. *J Neuropsychiatry. Clin Neurosci.* 1994; 4:348–357. [PubMed: 7841806]
- Goldman-Rakic PS, Porrino LJ. The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. *J Comp Neurol.* 1985; 242:535–560. [PubMed: 2418080]
- Gundersen HJG. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. *J Microsc.* 1986; 143:3–45. [PubMed: 3761363]
- Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *Acta Pathol Microbiol Immunol Scand.* 1988; 96:379–394.
- Gundersen HJG, Jensen EBV, Kieu K, Nielsen J. The efficiency of systematic sampling in stereology – reconsidered. *J Microsc.* 1999; 193:199–211. [PubMed: 10348656]
- Gur RE, Cowell PE, Latshaw A, Turetsky BI, Grossman RI, Arnold SE, Bilker WB, Gur RC. Reduced dorsal and orbital prefrontal gray matter volumes in schizophrenia. *Arch Gen Psychiatry.* 2000; 57:761–768. [PubMed: 10920464]
- Guttlerez-Galve L, Wheeler-Kingshott CAM, Altmann DR, Price G, Chu EM, Leeson VC, Lobo A, Barker GJ, Barnes TRE, Joyce EM, Ron MA. Changes in the frontotemporal cortex and cognitive correlates in first episode psychosis. *Biol Psychiatry.* 2010; 68:51–60. [PubMed: 20452574]
- Harms MP, Wang L, Campanella C, Aldridge K, Moffitt AJ, Kuelper J, Ratnanather JT, Miller MI, Barch DM, Csernansky JG. Structural abnormalities in gyri of the prefrontal cortex in individuals with schizophrenia and their unaffected siblings. *Brit J Psychiatry.* 2010; 196:150–157. [PubMed: 20118463]
- Hollister JM, Laing P, Mednick SA. Rhesus incompatibility as a risk factor for schizophrenia in male adults. *Arch Gen Psychiatry.* 1996; 53:19–24. [PubMed: 8540773]
- Insel BJ, Schaefer CA, McKeague IW, Susser ES, Brown AS. Maternal iron deficiency and the risk of schizophrenia in offspring. *Arch Gen Psychiatry.* 2008; 65:1136–1144. [PubMed: 18838630]
- Jones, EG. Connectivity of the primate sensory-motor cortex. In: Jones, EG.; Peters, A., editors. *Cerebral Cortex. Volume 5, Sensory-Motor Areas and Aspects of Cortical Connectivity*. Plenum Press; New York (NY): 1986. p. 113-183.
- Joshi SC, Miller MI. Landmark matching via large deformation diffeomorphisms. *IEEE Transactions Image Processing.* 2000; 9:1357–1370.
- Joyner AH, Roddey JC, Bloss CS, Bakken TE, Rimol LM, Melle I, Agartz I, Djurovic S, Topol EJ, Schork NJ, Andreassen OA, Dale AM. A common MECP2 haplotype associates with reduced cortical surface area in humans in two independent populations. *Proc Natl Acad Sci USA.* 2009; 106:15483–15488. [PubMed: 19717458]
- Lotfipour S, Ferguson E, Leonard G, Perron M, Pike B, Richer L, Seguin JR, Toro R, Veillette S, Pausova Z, Paus T. Orbitofrontal cortex and drug use during adolescence. Role of prenatal exposure to maternal smoking and BDNF genotype. *Arch Gen Psychiatry.* 2009; 66:1244–1252. [PubMed: 19884612]
- Lopez-Garcia P, Aizenstein HJ, Snitz BE, Walter RP, Carter CS. Automated ROI-based brain parcellation analysis of frontal and temporal brain volumes in schizophrenia. *Psychiatry Res.* 2006; 147:153–156. [PubMed: 16949259]
- Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, Biechele T, Petryshen TL, Moon RT, Haggarty SJ, Tsai LH. DISC1 regulated neural progenitor proliferation via modulation of GSK3 β /catenin signaling. *Cell.* 2009; 136:1017–1031. [PubMed: 19303846]
- Mathalon DH, Sullivan EV, Rawles JM, Pfefferbaum A. Correction for head size in brain-imaging measurements. *Psychiatry Res.* 1993; 50:121–139. [PubMed: 8378488]
- McConnell SK. Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation. *J Neurosci.* 1988; 8:945–974. [PubMed: 3346731]

- Miller MI, Massie AB, Ratnanather JT, Botteron KN, Csernansky JG. Bayesian construction of geometrically based cortical thickness metrics. *Neuroimage*. 2000; 12:676–687. [PubMed: 11112399]
- Miller MI, Hosakere M, Barker AR, Priebe CE, Lee N, Ratnanather JT, Wang L, Gado M, Morris JC, Csernansky JG. Labeled cortical mantle distance maps of the cingulate quantify differences between dementia of the Alzheimer type and healthy aging. *Proc Natl Acad Sci USA*. 2003; 100:15172–15177. [PubMed: 14657370]
- Pakkenberg B. Total nerve cell number in neocortex in chronic schizophrenic and controls estimated using optical disectors. *Biol Psychiatry*. 1993; 34:768–772. [PubMed: 8292680]
- Pannizzon MS, Fennema-Notestine C, Eyer LT, Jernigan TL, Prom-Wormley E, Neale M, Jacobson K, Lyons MJ, Grant MD, Franz CE, Xian H, Tsuang M, Fischl B, Seidman L, Dale A, Kremen WS. Distinct genetic influences on cortical surface area and cortical thickness. *Cereb Cortex*. 2009; 19:2728–2735. 2009. [PubMed: 19299253]
- Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex. II. Variability in locations of areas 9 and 46 and relationship to the Talairach Coordinate System. *Cereb Cortex*. 1995; 5:323–337. [PubMed: 7580125]
- Rakic P. Specification of cerebral cortical areas. *Science*. 1988a; 241:170–176. [PubMed: 3291116]
- Rakic P. Defects of neuronal migration and the pathogenesis of cortical malformations. *Prog Brain Res*. 1988b; 73:15–37. [PubMed: 3047794]
- Rakic P. A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci*. 1995; 18:383–388. [PubMed: 7482803]
- Rakic P, Suner I, Williams RW. A novel cytoarchitectonic area induced experimentally within primate visual cortex. *Proc Natl Acad Sci USA*. 1991; 88:2083–2087. [PubMed: 2006147]
- Rakic P, Ayoub AE, Breunig JJ, Dominguez MH. Decision by division: Making cortical maps. *Trend Neurosci*. 2009; 32:291–301. [PubMed: 19380167]
- Schindler MK, Wang L, Selemon LD, Goldman-Rakic PS, Csernansky JG. Abnormalities of thalamic volume and shape detected in fetally irradiated rhesus monkeys with high dimensional brain mapping. *Biol Psychiatry*. 2002; 51:827–837. [PubMed: 12007457]
- Selemon LD, Rajkowska G, Goldman-Rakic PS. Abnormally high neuronal density in the schizophrenic cortex: A morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry*. 1995; 52:805–818. [PubMed: 7575100]
- Selemon LD, Rajkowska G, Goldman-Rakic PS. Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: Application of a 3-dimensional, stereologic counting method. *J Comp Neurol*. 1998; 392:402–412. [PubMed: 9511926]
- Selemon LD, Mrzljak J, Kleinman JE, Herman MM, Goldman-Rakic PS. Regional specificity in the neuropathologic substrates of schizophrenia: A morphometric analysis of Broca's area 44 and area 9. *Arch Gen Psychiatry*. 2003; 60:69–77. [PubMed: 12511174]
- Selemon LD, Wang L, Nebel MB, Csernansky JG, Goldman-Rakic PS, Rakic P. Direct and indirect effects of fetal irradiation on cortical gray and white matter volume in the macaque. *Biol Psychiatry*. 2005; 57:83–90. [PubMed: 15607304]
- Selemon LD, Begovic' A, Rakic P. Selective reduction of neuronal number and volume of the mediodorsal nucleus of the thalamus in macaques following irradiation at early gestational ages. *J Comp Neurol*. 2009; 515:454–464. [PubMed: 19459221]
- Sidman RL, Rakic P. Neuronal migration, with special reference to developing human brain: a review. *Brain Res*. 1973; 62:1–35. [PubMed: 4203033]
- Sinclair WK, Morton RA. X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells. *Radiat Res*. 1966; 29:450–474. [PubMed: 5924188]
- Stolp HB, Turnquist C, Dziegielewska KM, Saunders NR, Anthony DC, Molnar Z. Reduced ventricular proliferation in the foetal cortex following maternal inflammation in the mouse. *Brain*. 2011; 134:3236–3248. [PubMed: 21964917]
- Stolp H, Neuhaus A, Sundramoorthi R, Monar Z. The long and short of it: Gene and environmental interactions during early cortical development and consequences for long term neurological disease. *Front Psychiatry*. 2012; 3:50. [PubMed: 22701439]

- Sullivan EV, Lim KO, Mathalon D, Marsh L, Beal DM, Harris D, Hoff AL, Faustman WO, Pfefferbaum A. A profile of cortical gray matter volume deficits characteristic of schizophrenia. *Cereb Cortex*. 1998; 8:117–124. [PubMed: 9542891]
- Sun D, Stuart GW, Jenkinson M, Wood SJ, McGorry PD, Velakoulis D, van Ert TGM, Thompson PM, Toga AW, Smith DJ, Cannon TD, Pantelis C. Brain surface contraction mapped in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. *Mol Psychiatry*. 2009; 14:976–986. [PubMed: 18607377]
- Suner I, Rakic P. Numerical relationship between neurons in the lateral geniculate nucleus and primary visual cortex in macaque monkeys. *Vis Neurosci*. 1996; 13:585–590. [PubMed: 8782386]
- Susser ES, Lin SP. Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944–1945. *Arch Gen Psychiatry*. 1992; 49:983–988. [PubMed: 1449385]
- Toro R, Leonard G, Lerner JV, Lerner RM, Perron M, Pike GB, Richer L, Velette S, Pausova Z, Paus T. Prenatal exposure to maternal cigarette smoking and the adolescent cerebral cortex. *Neuropsychopharmacol*. 2008; 33:1019–1027.
- Walhovd KB, Moe V, Slinning K, Due-Tønnessen P, Bjørnerud A, Dale AM, van der Kouwe A, Quinn BT, Kosofsky B, Greve D, Fischl B. Volumetric cerebral characteristics of children exposed to opiates and other substances *in utero*. *Neuroimage*. 2007; 36:1331–1344. [PubMed: 17513131]
- Walker AE. A cytoarchitectural study of the prefrontal area of the macaque monkey. *J Comp Neurol*. 1940; 73:59–86.
- Weinberger DR, Berman KF, Illowsky BP. Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia: I. Regional cerebral blood flow (rCBF) evidence. *Arch Gen Psychiatry*. 1986; 43:114–124. [PubMed: 3947207]
- Zhang Y, Wu Y, Zhu M, Wang C, Wang J, Zhang Y, Yu C, Jiang T. Reduced cortical thickness in mental retardation. *PLoS ONE*. 2011; 6:329673. doi:10.1371/journal.pone.0029673.
- Zhou D, Level C, Lepage C, Rasmussen C, Evans A, Wyper K, Pei J, Andrew G, Massey A, Massey D, Beaulieu C. Developmental cortical thinning in fetal alcohol spectrum disorders. *Neuroimage*. 2011; 58:16–25.

Figure 1.

Cytoarchitectonic delineation of area 46 and segmentation of anatomic boundaries for transfer to MRI images. (A) Low power photomicrograph of a coronal section of the prefrontal cortex with boxes indicating high power views shown in B–D. (B) The cytoarchitectonic characteristics of area 46 are illustrated, and borders of layer I, II–IV, and V–VI are shown. (C) The lateral border of area 46 with adjacent area 12 and (D) the medial border of area 46 with area 9 are indicated on high power views. (E) Segmentation of gray matter in the whole coronal section is indicated by large dots. Laminar borders are indicated by smaller dots. The boundaries of area 46 with neighboring cortical areas are marked by bars. (F) Area 46 with color-coded lamina is shown extracted for transfer to the corresponding MR slice. Red = layer I, green = layers II–IV, blue = layers V–VI. Scale bar = 1 mm for A,E,F; scale bar = 100 μ m for B,C,D.

Figure 2.

Measurements of area 46 volume, surface area, and cortical thickness in MRI volumes. (A) Area 46 volume was decreased by 20% in early gestationally irradiated animals (EGAs) and 43% in midgestationally irradiated animals (MGAs) in comparison to controls (CONs). (B) Surface area was decreased by 19% in EGAs and 40% in MGAs relative to CONs. (C) Cortical thickness was reduced only in MGAs (13%) in comparison to CONs. Open diamonds = non-irradiated controls (nCONs); Open circles = sham-irradiated controls (shCONs); Filled circles = EGAs; Filled diamonds = MGAs. * $p < 0.05$; *** $p < 0.005$ indicates significant difference relative to CONs (nCONs + shCONs).

Figure 3.

Graphs illustrating the selective involvement of supragranular layers in area 46 pathology in MGAs. (A) Measurement of laminar volume indicated that volume was reduced in MGAs in layers I (43%) and II–IV (62%) compared to CONs. Volume of layers II–IV (23%) and V–VI (17%) was reduced in EGAs compared to CONs. (B) In MGAs laminar thickness was reduced only in layer II–IV (28%). Laminar thickness did not differ for any of the three layers in EGAs compared to CONs. Open diamonds = nCONs; Open circles = shCONs; Filled circles = EGAs; Filled diamonds = MGAs. * $p < 0.05$; *** $p < 0.005$, **** $p < 0.001$ indicates significant difference relative to CONs (nCONs + shCONs).

Figure 4.

Photomicrographs illustrating the pathology in supragranular layers in area 46 in MGAs. (A) A cell-sparse stratum (arrows) in layer III is evident throughout the PFC on a low power coronal section from an MGA. (B) The cytoarchitecture of area 46 in an nCON brain is shown in comparison to (C) cytoarchitecture of area 46 in an MGA. Note that layer II–IV is thinner in the MGA cortex and that a cell sparse band (arrowheads) runs through this layer. Cytoarchitecture in the EGA brains did not exhibit overt pathology (see Fig. 1B). Scale bar = 1 mm for A; scale bar = 100 μ m for B,C.

Figure 5.

Stereologic measurement of total neuron and glia number in area 46 did not uncover significant differences in number of neurons and glia across the three groups. Note that additional nCONs that had not been imaged were included in the postmortem stereologic analyses. Open diamonds = nCONs; Open circles = shCONs; Filled circles = EGAs; Filled diamonds = MGAs.

Figure 6.

Neuronal density plots for Cohort 2. Mean neuronal density was 21% higher in EGAs compared to CONs. Open diamond = nCON; Open circles = shCONs; Filled circles = EGAs;. * $p < 0.05$; indicates significant difference relative to CONs (nCON + shCONs).

Table 1

Radiation Exposure and Dosage

Animal	Sex	Age (yrs) at scan	Age (yrs) at sac	Time in embryonic day (E) and dose of radiation (cGy)	Total dose (cGy)
Cohort 1					
nCON-Cr	M	7.92	9.42		0
nCON-Sa	F	9.92	10.00		0
nCON-Me	F	8.33	12.42		0
nCON-Li	F	N/A	4.83		0
nCON-Si	F	N/A	7.58		0
nCON-Mu	F	N/A	14.17		0
EGA-Ro	M	5.50	7.71	E33(25), E35(50), E37(50), E41(50)	175
EGA-Ne	M	5.42	6.42	E33(50), E35(50), E36(50), E37(50)	200
EGA-Na	F	6.75	7.13	E35(50), E37(50), E39(50), E42(50)	200
MGA-Ob	M	8.17	9.75	E73(100), E74(100), E75(100)	300
MGA-Pi	M	8.17	11.50	E73(100), E74(100), E75(100)	300
MGA-Al	F	9.25	11.96	E70(100), E72(100), E75(100) E77(100), E79(100), E81(100)	600
Cohort 2					
shCON-Ma	M	5.75	5.83	4 doses ketamine-E29, E32, E34, E36	0
shCON-Ot	M	5.92	6.04	2 doses ketamine-E34, E36	0
shCON-Vi	F	5.50	5.58	3 doses ketamine-E39, E41, E43	0
shCON-La	F	5.13	5.25	4 doses ketamine-E32, E34, E37, E39	0
nCON-Ju	F	5.56	5.67		0
EGA-Bu	M	5.67	5.67	E35(50), E37(50), E38(50)	150
EGA-Aj	M	5.08	5.17	E33(50), E35(50), E37(50)	150
EGA-Tr	M	5.92	5.96	E34(50), E36(50), E38(50), E41(50)	200
EGA-Sa	F	5.92	5.96	E32(50), E34(50), E36(50)	150
EGA-Lo	F	6.00	6.08	E30(50), E33(50), E35(50), E37(50)	200

EGA= early gestationally irradiated animal; MGA = midgestationally irradiated animal; nCON= non-irradiated control; shCON = sham-irradiated control, sac = sacrifice

Table 2

Means (SD) and Statistics

Parameter	CON	EGA	MGA	Statistic	Pairwise Comparisons *
Age at scan (yrs)					
Cohort 1	8.72 (1.06)	5.89 (0.75)	8.53 (0.62)	F(2,6)=10.94 p=0.010	E<C (0.017) M=C (1.000) M>E (0.024)
Cohort 2	5.57 (0.30)	5.72 (0.38)		F(1,8)= 0.46 p = 0.516	
Combined cohorts	6.75 (1.75)	5.78 (0.50)	8.53 (0.62)	F(2,16)= 5.63 p = 0.014	E=C (0.391) M=C (0.140) M>E (0.013)
Age at sac (yrs)					
Cohort 1	9.74 (3.34)	7.09 (0.65)	11.07 (1.17)	F(2,9)= 1.19 p= 0.204	
Cohort 2	5.67 (0.29)	5.77 (0.37)		F(1,8)= 0.20 p= 0.667	
Combined cohorts	7.89 (3.18)	6.29 (0.84)	11.07 (1.17)	F(2,19)=4.47 p = 0.026	E=C (0.477) M=C (0.165) M>E (0.023)
Volume (mm ³)	645.32 (70.94)	516.81 (114.89)	365.36 (20.98)	F(2,16)=11.43 p=0.001	E<C (0.033) M<C (0.001) M E (0.072)
Surface area (mm ²)	317.46 (33.74)	256.90 (56.94)	190.60 (1.55)	F(2,16)= 9.98 p=0.002	E<C (0.041) M<C (0.002) M=E (0.120)
Thickness (mm)	2.99 (0.20)	3.01 (0.18)	2.59 (0.16)	F(2,16)= 5.83 p= 0.013	E=C (1.000) M<C (0.020) M<E (0.016)
Volume (mm ³)					
Layer I	98.12 (11.78)	80.48 (17.96)	55.89 (9.10)	F(2,16)= 9.58 p=0.002	E C (0.083) M<C (0.002) M E (0.079)
Layer II–IV	321.45 (43.16)	248.89 (66.34)	123.69 (11.58)	F(2,16)=15.76 p<0.001	E<C (0.042) M<C (<0.001) M<E (0.008)
Layer V–VI	225.75 (20.67)	187.44 (33.54)	185.78 (3.08)	F(2,16)= 5.12 p=0.019	E<C (0.029) M=C (0.114) M=E(1.000)
Thickness (mm)					
Layer I	0.73 (0.09)	0.66 (0.05)	0.64 (0.18)	F(2,16)= 1.52 p=0.249	
Layer II–IV	1.44 (0.22)	1.37 (0.17)	1.04 (0.07)	F(2,16)= 5.35 p=0.017	E=C (1.000) M<C (0.016) M<E (0.049)
Layer V–VI	2.01 (0.22)	2.00 (0.23)	1.93 (0.12)	F(2,16)= 0.17 p=0.843	
Neurons (10 ⁷)	3.69 (0.66)	3.09 (1.13)	2.31 (0.18)	F(2,19)= 3.50 p=0.051	E=C (0.420) M C (0.064) M=E (0.570)
Glia (10 ⁷)	2.98 (0.53)	2.55 (1.12)	2.00 (0.10)	F(2,19)= 2.06 p=0.115	
Neuron/glia ratio	1.24 (0.12)	1.24 (0.14)	1.16 (0.05)	F(2,19)= 0.60 p=0.561	
Neuronal density (10 ³ /mm ³)					
Cohort 1	61.85 (9.42)	53.91 (8.14)	61.94 (5.36)	F(2,5)= 0.24 p=0.795	
Cohort 2	52.40 (2.40)	63.62 (9.30)		F(1,7)= 5.66 p= 0.049	
Glial density (10 ³ /mm ³)	47.75 (6.54)	48.07 (11.49)	54.87 (4.49)	F(2,16)= 0.78 p=0.476	
Percent total GM	1.38 (0.12)	1.28 (0.17)	1.10 (0.16)	F(2,14)= 3.42 p=0.062	E=C (0.665) M C (0.062) M=E (0.330)

Parameter	CON	EGA	MGA	Statistic	Pairwise Comparisons*
Tot GM (10 ³ mm ³)	48.46 (3.20)	40.32 (5.79)	33.59 (3.89)	F(2,14)=10.76 p=0.001	E<C (0.020) M<C (0.002) M=E (0.165)
MD neurons (10 ⁶)	1.74 (0.21)	1.54 (0.27)		F(1,17)= 3.15 p=0.094	

CON or C = control; EGA or E = early gestationally irradiated animal; GM= gray matter; MD = medial dorsal nucleus; MGA or M = midgestationally irradiated animal; sac = sacrifice; Tot GM = total gray matter

* represent Bonferroni corrected p values; Bold indicates significant p values; and indicate trend level p values.