Cranial Irradiation Alters the Behaviorally Induced Immediate-Early Gene Arc (Activity-Regulated Cytoskeleton-Associated Protein)

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Abstract
Therapeutic irradiation of the brain is commonly used to treat brain tumors but can induce cognitive impairments that can severely affect quality of life. The underlying mechanisms responsible for radiation-induced cognitive deficits are unknown but likely involve alterations in neuronal activity. To gain some mechanistic insight into how irradiation may affect hippocampal neurons known to be associated with cognitive function, we quantitatively assessed the molecular distribution of the behaviorally induced immediate-early gene Arc (activity-regulated cytoskeleton-associated protein) at the level of mRNA and the protein. Young adult C57BL/6J mice received whole-brain irradiation with 0 or 10 Gy, and 1 week or 2 months later, exploration of a novel environment was used to induce Arc expression. The fractions of neurons expressing Arc mRNA and Arc protein were detected using fluorescence in situ hybridization and immunocytochemistry, respectively. Our results showed that there was a significant reduction in the percentage of neurons expressing Arc protein 1 week after irradiation, whereas 2 months after irradiation, there was a reduction in the percentage of neurons expressing both Arc mRNA and Arc protein. Importantly, radiation-induced changes in Arc expression were not a result of neuronal cell loss. The changes observed at 2 months were associated with a significant increase in the number of activated microglia, supporting the idea that inflammation may contribute to neuronal dysfunction. These findings are the first to show that local brain irradiation initiates changes in hippocampal neurons that disrupt the activity patterns (Arc expression) associated with neuroplasticity and memory.

Introduction
The brain is exposed to ionizing irradiation during the management of brain tumors, and the dose that can be administered safely is largely dictated by the tolerance of normal tissues surrounding the tumor (1). In the United States, a large number of patients (200,000–300,000/y) with primary or metastatic tumors in the brain will require large volume or whole-brain irradiation (2), and in at least some of these patients, there is a strong likelihood of developing adverse reactions in terms of cognitive decline (3, 4). In fact, it has been recently reported that after irradiation, neurocognitive function changes correlate with alterations in quality of life that in turn correlate with median survival (5). Currently, there are no successful long-term treatments or preventive strategies for radiation-induced cognitive impairments (6–8). A better understanding of the cellular and molecular factors associated with the development of cognitive injury is essential to the management of this serious complication of cranial radiotherapy.

Radiation injury can involve multiple regions and cell/tissue types, and a large number of physical and biological factors influence the expression and extent of damage (9, 10). In patients, overt tissue injury generally occurs after relatively high doses (>60 Gy, fractionated), and the morphologic consequences of such exposures are well-documented in humans and experimental systems (1, 9, 10). In humans, less severe morphologic changes can occur after relatively lower doses (e.g., as low as 20 Gy fractionated), resulting in variable degrees of cognitive impairment (11–13). Such impairment has a diverse character, but often includes hippocampal-dependent functions involving learning, memory, and spatial information processing. Recently, this has prompted consideration of whether or not strategies should be implemented to specifically shield or reduce hippocampal exposure during radiotherapy (5). A number of animal studies have been performed that confirm the importance of hippocampus-related effects in the evolution of radiation-induced cognitive injury (14–17). Although those laboratory studies involved varying doses, delivery schemes (e.g., fractionation), end points, and rodents of various ages, they provide compelling evidence that relatively low radiation doses cause hippocampus-dependent cognitive impairments without necessarily inducing overt signs of tissue destruction.

The cellular and molecular mechanisms underlying radiation-induced cognitive impairments are still not known, but almost certainly involve changes associated with neuronal function, either through direct cell damage or damage mediated through factors from the irradiated microenvironment. Information is starting to appear regarding gene expression changes in brain cells after exposure to ionizing irradiation (reviewed in ref. 18) but most of the available experimental data relate to very early changes (<1–24 h; refs. 19, 20), and there is only one consideration of a gene (c-fos) specifically known to be linked to learning and memory (20). Gene expression induced during learning produces proteins that alter the composition of neuronal networks and provide a mechanism for translating synaptic plasticity into changes in synaptic strength (memory); a number of activity-regulated genes have been identified for this function (21). Although immediate-early genes (IEG) like c-fos and zif268 are involved in mechanisms associated with the maintenance of memory (22), activity-regulated cytoskeleton-associated protein (Arc) is an activity-induced gene that correlates both temporally and spatially with the stimulus that induced its transcription (23). Arc is induced in hippocampal and parietal...
neurons after a behavioral experience (24), and Arc protein plays a critical role in the maintenance phase of long-term potentiation, and spatial memory consolidation (25). Furthermore, the reduction of Arc expression, either genetically (26) or with antisense oligonucleotides (25), results in cognitive impairment with respect to long-term memory formation. Perhaps most importantly, Arc is the only known IEG whose mRNA moves rapidly to the dendrites closest to active synapses where it is locally translated (23). Taken together, this information provides a mechanistic link between Arc and hippocampal-dependent function. Furthermore, it provides a strong rationale for using Arc expression to assess specific neuronal activities associated with cognitive impairments and if those activities are altered by cranial irradiation. It is relevant to this argument that Alzheimer's disease patients with pronounced activities are altered by cranial irradiation. It is relevant to this argument that Alzheimer's disease patients with pronounced deficits in hippocampus-dependent memory functions have an almost 3.5-fold lower baseline level of Arc expression when compared with non–Alzheimer's patients (27). These data support the general hypothesis that reduced Arc expression in the hippocampus is associated with cognitive impairments.

We contend that a better understanding of the cellular and molecular mechanisms underlining radiation-induced cognitive impairment is critical for the development of approaches to manage this potentially serious effect. Thus, the present study was done to provide a novel insight into how ionizing irradiation affects a specific neuronal function (Arc expression) that is known to be associated with cognitive performance. This type of information is currently unavailable and is essential when trying to ascertain the risk(s) of specific central nervous system–related effects and develop potential strategies to manage radiation-induced cognitive dysfunction.

Materials and Methods

Animals. A total of 56 2-month-old male C57BL/6J mice were used in these studies; 12 for a pilot study of the characterization of Arc expression, and 48 for the study of radiation effects. Animals were purchased from a commercial vendor (Jackson Laboratory), and all animal care and use was conducted in accordance with the U.S. Department of Health and Human Services Guide for the Care and Use of Laboratory Animals, and institutional guidelines for care and handling of laboratory animals. To minimize any stress associated with group-housing that could affect our end points, all animals were housed singly in Plexiglas cages with free access to food and water and maintained on a 12/12 h light/dark cycle in a temperature-controlled room (22 °C). Animals were handled for 5 min once a day for 10 days before the behavioral experiment to familiarize them to the experimenter and to the handling procedures.

Pilot study. To characterize the molecular distribution of the IEG Arc at the level of mRNA and protein in dentate granule neurons (Fig. 1), 12 mice were randomly separated into two groups of 6; caged controls and animals that were allowed to explore a novel environment (23). The behavioral procedure involved an initial 5-min exploration of a novel environment, a 25-min rest period, and then a second 5-min exploration of the same environment. During the interval between the two exploration sessions, animals remained undisturbed in their own cages. The novel environment was an open, white acrylic box measuring 61 × 61 cm with 20-cm-high walls that contain plastic chews and a polycarbonate house. To exclude the possibility that deficits in IEG expression resulted from altered exploratory behavior and consequent lack of sensory stimulation, motor activity of the mice was visually monitored by an operator blind to animal identity. Light intensity and distal and local spatial cues were maintained throughout both behavioral explorations. Immediately after the second 5-min exploration, mice were deeply anesthetized and killed by decapitation. The brain was quickly removed (within 60 s) and frozen in −70 °C isopentane. Mice from the caged control group remained undisturbed in their cage until euthanasia.

Irradiation. For irradiation, mice were anesthetized using an i.p. injection of ketamine (60 mg/kg) and medetomidine (0.25 mg/kg). Irradiation with a Phillips orthovoltage X-ray system was done as previously described (15, 28). Briefly, a special positioning jig was used so four animals could be irradiated simultaneously; the heads were centered in a 5 × 6 cm2 field. The incident beam was directed down onto the head and a special lead shield was used to cover the eyes, nose, cerebellum, and the body.

Figure 1. Qualitative characterization of Arc expression in granule cell neurons of the DG. Arc was induced by two 5-min behavioral explorations of a novel environment that were separated by 25 min. Intranuclear foci of Arc mRNA were induced by the second exploration, ~5 min before tissue collection, and were detected using fluorescent in situ hybridization (A). Cytoplasmic Arc mRNA (B) or Arc protein (C) were detected in neurons activated by the first exploration. Both nuclear foci (second exploration, short yellow arrows) and cytoplasmic Arc mRNA (first exploration, long white arrow) were seen in ~90% of cells immunoreactive for Arc (D). Digoxigenine-labeled Arc antisense probe was detected with CY3 (red, A, B, and D), and immunofluorescence staining detected Arc protein (red, C). Cell nuclei were counterstained (green); magnification for all images, ×63.
Dosimetry was done using a Keithley electrometer ionization chamber as previously described (28). Unirradiated mice were anesthetized at the same time as those who received a single dose of 10 Gy.

Behavioral procedures. At specified times after irradiation (7 days or 2 months), mice from each treatment cohort (0 or 10 Gy) were randomly assigned to two groups of six mice; exploration and caged controls. The animals were treated exactly as described above for the pilot study.

Histologic procedures. Frozen brains (kept in –70°C) were divided at the midline, and one hemisphere from each of the six animals from a given experimental group were blocked together, cryosectioned, and placed on a glass microscopic slide (29). Brain sections were taken from the medial portion of the dorsal hippocampus (anteroposterior, –2.92–4.0 mm from the bregma). Thus, tissues from all the individuals in a given group were processed and stained simultaneously. All slides were stored at –70°C until processing for immunocytochemistry or in situ hybridization.

Fluorescence in situ hybridization. Four to six slides from each treatment group, each containing sections from all the mice in that group, were prepared for fluorescence in situ hybridization. Arc mRNA (Figs. 1A,B,D and 2B), and zif268 and c-fos mRNA (Fig. 2C and D) were detected as previously reported in detail (23, 29). Briefly, hapten-labeled antisense riboprobes were hybridized together with the tissues overnight. The digoxigenin-labeled Arc full-probe riboprobe was detected with anti-digoxigenin-HRP conjugate (Roche) and revealed with a cyanine-3 (CY3) substrate kit. Nuclei were counterstained with sytox-green (Molecular Probes).

Immunofluorescence staining. Four to six slides from each treatment group were also used for immunohistochemical staining of Arc protein; the sections used were adjacent to those used for fluorescence in situ hybridization. Quantitative assessment was done using methods we have previously reported in detail (29, 30). Brieﬂy, tissues were ﬁxed in 2% paraformaldehyde, and after blocking with a tyramide signal ampliﬁcation kit (Perkin-Elmer Life Science), were incubated in polyclonal rabbit anti-Arc antibody for 48 h at 4°C (rabbit anti-Arc, a gift from S. Chowdhury and P.F. Worley, Johns Hopkins University School of Medicine, Baltimore, MD). Sections were then incubated with an antirabbit biotinylated secondary antibody (Vector Laboratories) for 2 h at room temperature, followed by amplification with an avidin-biotin system for 45 min. Arc protein staining was revealed using a CY3 tyramide signal amplification ﬂuorescence system (Perkin-Elmer Life Science; Fig. 1C). For staining of activated microglia, after ﬁxation in paraformaldehyde and the subsequent blocking step (29–31), the primary monoclonal rat anti-mouse CD68 antibody (Serotec, Inc.) was applied overnight followed by a secondary biotinylated antimouse antibody (Vector Laboratories). Activated microglia were labeled with CY3 and nuclei were counterstained with sytox-green (Molecular Probes).

Microscopy, image acquisition, and analysis. Microscopic imaging for Arc mRNA, Arc protein, and activated microglia was performed using a Zeiss AXIO IMAGER Z1 microscope with motorized Z-drive for transmitted light and epifluorescence (31). For each end point, the four to six coronal sections per mouse were used to reconstruct mosaics of the entire hippocampal dentate gyrus (DG; ref. 30). Each mosaic was formed from four to ﬁve Z-stack (1.0 μm optical thickness/plane) images. Contrast and intensity variables for Arc, zif268, and c-fos were set using the tissue sections from the caged controls only. For consistency, these variables were kept constant for the rest of the sections on the slide.

Image analysis. Manual cell counts of cells expressing Arc mRNA and Arc protein were performed by an experimenter blind to the relationship between the experimental conditions they represented (i.e., sham, irradiated, caged control, exploration; refs. 29, 30). Arc mRNA–positive and Arc protein–positive neurons were identiﬁed when the staining constituted at least 60% of the cell body (Fig. 1) and was detectable throughout three planes across the Z-stack. To avoid classiﬁcation errors, we carefully veriﬁed that the staining belonged to the cell of interest by checking the nuclear counterstaining. The number of cells/mm2 charac- terized by these criteria were determined. The total number of neurons per DG was estimated using a correction factor which was derived from the 24 different Z-stacks (20× magniﬁcation) from representative mice from

Figure 2. mRNA expression (red) of the IEG Arc (B), zif268 (C), and c-fos (D) within the granule cell layer of the DG after behavioral exploration. Caged control animals showed low expression of IEGs (A). Bar, 100 μm.
both irradiated and nonirradiated treatment groups. The total number of neurons per stack was counted and the area in the granule cell layer (in mm²) from the middle plane was calculated. Using this factor, we calculated the percentage of neurons expressing Arc mRNA and Arc protein for each mouse (29, 30). The total number of neurons analyzed for each mouse averaged ~2,000. The total number of activated microglia (CD68 positive) within the DG and hilar region of the hippocampus was manually counted and divided for the area (in mm²), as reported previously (31).

**Statistical methods and sample size.** StatView software (version 5.0.1) was used to perform one-way ANOVA tests. The caged control (0 and 10 Gy) and behaviorally tested groups (0 and 10 Gy) were the independent variables, and the percentages of positive cells, from various categories described above, were the dependent variables. When an overall ANOVA was significant (P < 0.05), individual between-group comparisons were performed with Bonferroni post hoc tests to correct for multiple comparisons.

**Results**

The aim of the pilot study was the characterization of the molecular distribution of the IEG Arc at the level of mRNA and protein in the dentate granule neurons. Similar to what was observed previously in rats (23), neurons expressing only intranuclear foci Arc (Fig. 1A) represented cells activated within ~5 minutes of euthanasia (i.e., second exploration). Neurons showing only cytoplasmic Arc mRNA (Fig. 1B) or Arc protein (Fig. 1D) were active for ~30 minutes before the animal was euthanized (i.e., first exploration). Cells showing both Arc foci and cytoplasmic Arc mRNA (Fig. 1D) represented neurons that were activated during both explorations, and constituted ~90% of the Arc-positive neurons in the granule cell layer of the DG. In caged control animals, there were few neurons expressing Arc mRNA and Arc protein. Exploration of a novel environment also resulted in the concomitant expression of the IEGs zif268 and c-fos (Fig. 2). Overall, given the well-described characterization of Arc expression (23, 24, 29) and its relationship to learning and memory (see Introduction), we focused our radiation studies solely on the expression of the IEG Arc.

Whole-brain irradiation with 10 Gy was tolerated by all animals; none were lost and all mice gained weight normally over the duration of the study. All mice that explored the novel environment (irradiated and nonirradiated) did so in a similar manner (data not shown), indicating that there were no deficits in motor or exploratory behavior as a result of brain irradiation. To assure that the measures of Arc mRNA and Arc protein were not influenced by radiation-induced decreases in neuronal cell number, we quantified neurons in the dentate granule cell layer 2 months after irradiation. In nonirradiated mice, there were 1,502 ± 351 neurons/mm² (mean ± SE), whereas after 10 Gy, there were 1,227 ± 191; the difference was not significant.

Caged control nonirradiated mice showed only few granule cells expressing Arc mRNA (Fig. 3A) and Arc protein (data not shown). Quantitatively in these mice, the fractions of neurons expressing Arc mRNA averaged <1% at both postirradiation time points (Fig. 4A and C). The fractions of neurons expressing Arc protein were slightly higher (Fig. 4A and C). In nonirradiated mice that did explore the novel environment, there were more neurons expressing Arc mRNA (Fig. 3B) and protein (data not shown). In those mice, the percentages of neurons that expressed Arc mRNA and protein were similar and were increased 5-fold to 6-fold relative to caged controls at both postirradiation time points [Fig. 4A and C; ANOVA, Arc mRNA: F(1, 10) = 54.8, P < 0.001, 1 week; F(1, 10) = 69.24, P < 0.001, 2 months; ANOVA, Arc protein: F(1, 10) = 42.5; P < 0.001, 1 week; F(1, 10) = 23; P < 0.001, 2 months].

At both times after irradiation, the fractions of neurons expressing Arc mRNA and Arc protein in caged control mice were similar to nonirradiated animals (Fig. 4B and D), indicating that radiation alone did not affect the basal expression levels of Arc. In irradiated animals that did explore the novel environment, there were significant increases in the percentages of neurons expressing Arc mRNA and Arc protein relative to irradiated caged controls at 1 week after irradiation [ANOVA, Arc mRNA: F(1, 10) = 22.5; P < 0.001; ANOVA, Arc protein: F(1, 10) = 40.5; P < 0.001], but not at 2 months [ANOVA, F(1, 10) = 2.76; P < 0.12, Arc mRNA; ANOVA, F(1, 10) = 1.23; P < 0.29, Arc protein; Fig. 4B and D].

When irradiated and nonirradiated mice that explored the novel environment were compared at 1 week, there was no significant difference in the fraction of neurons expressing Arc mRNA (Fig. 5A), but there was a significant decrease in the fraction of neurons expressing Arc protein [ANOVA, F(1, 10) = 9.08; P < 0.013, for Arc protein; Fig. 5B]. Two months following brain irradiation, there were clear qualitative reductions in the number of neurons expressing Arc mRNA (Fig. 5C) and Arc protein (data not shown), and the fractions of neurons expressing Arc mRNA (Fig. 5C) or Arc protein (Fig. 5D) were significantly reduced compared with nonirradiated animals [ANOVA, Arc mRNA: F(1, 10) = 7.8; P < 0.019; and ANOVA, Arc protein: F(1, 10) = 6.42; P < 0.029].

![Figure 3](image-url) **Figure 3.** Arc mRNA expression (red) in caged control animals (A), nonirradiated animals that explored a novel environment (B), and in irradiated animals that explored a novel environment (C). Nuclei were counterstained (blue) in the fluorescent in situ hybridization analysis. Bar, 100 μm.
We have shown that the expression of behaviorally induced Arc mRNA and Arc protein are influenced by the presence of activated microglia (29, 30), the intrinsic brain inflammatory cells that are increased after irradiation (15, 28, 31, 32). Thus, we quantified the total number of activated microglia in the DG of mice that received 0 or 10 Gy. Immunofluorescent staining for CD68 showed that 1 week after irradiation, there was no significant increase in the total number of activated microglia/mm² in the DG compared with nonirradiated animals (data not shown). In contrast, at 2 months after irradiation, there were significantly more activated microglia as compared with nonirradiated animals [ANOVA, F(1, 22) = 4.56; P < 0.001].

Discussion

Therapeutic irradiation can induce cognitive impairments without necessarily causing the gross histologic disruption classically associated with exposure to high radiation doses (1). Given that postmitotic neurons are generally considered to be relatively radioresistant, new approaches/techniques have been used to identify other “targets” that may ultimately contribute to the pathogenesis of radiation-induced cognitive injury. Data now exist regarding neurogenesis (14, 15), specific genetic factors (33) or receptor expression (34), and show that changes in these end points can be associated with subsequent cognitive impairments. Still, there is considerable uncertainty regarding how molecular and cellular events within specific neuronal populations are translated into changes that affect behavioral performance. Understanding such changes will be critical to the development of strategies or approaches necessary to prevent or treat the cognitive changes induced by therapeutic irradiation of the brain. To our knowledge, the data presented here are the first to show that ionizing irradiation initiates changes in hippocampal neurons that disrupt the activity patterns (i.e., Arc expression) associated with neuroplasticity and memory.

In the present study, we were interested in assessing the molecular distribution of Arc at the level of mRNA and protein after a single X-ray dose that is known to induce hippocampus-dependent cognitive impairments. Although cognitive impairments were not directly assessed in this study, we have considerable data in our mouse model showing that 3 months after single doses of 5 to 10 Gy, there are significant alterations in hippocampus-dependent spatial learning and memory but not in nonspatial learning and memory (15, 35). We have also reported similar findings in a gerbil model (14, 36), and other investigators have also shown cognitive impairments in rodent models using doses and posttreatment follow-up times generally similar to ours (6, 7).

The hippocampus is critical for the acquisition (learning), consolidation, and retrieval of declarative memories (reviewed in ref. 37). Given the physiologic properties of Arc as they relate to hippocampal-dependent functions (23, 24, 29), disruption of the expression of this IEG within hippocampal neurons possibly provides a mechanistic link between Arc expression and altered cognition. In fact, several lines of investigation clearly show that if Arc is reduced using either a genetic approach (26) or with antisense technology (25), animals fail to form long-term memories. The hippocampus has been shown to be sensitive to therapeutic irradiation (11), and although such exposure has been shown to acutely affect the expression of a number of genes in the hippocampal formation (20), in our study, basal (caged controls) levels of Arc mRNA or Arc protein were unchanged by a dose of 10 Gy (Fig. 4). Regardless of radiation treatment, increased Arc expression at the level of mRNA and protein was only elevated when animals were engaged in a learning experience induced by exploration, thus confirming that Arc is rapidly induced by neuronal activity associated with learning and memory. The magnitudes of behaviorally induced Arc expression were 5-fold to 6-fold higher in nonirradiated animals compared with caged controls and were still 3-fold to 5-fold higher in irradiated mice (Fig. 4). Other IEGs can also be induced by a learning experience (ref. 21; Fig. 2), but Arc is unique given its well-described temporal dynamics (24) because it is the most responsive IEG to specific behavioral demands (23), and because its mRNA moves to the site of active synapses in which it is locally translated (38).

Although the percentages of Arc-expressing neurons in the DG are fairly low (i.e., <10%), they represent a relatively large number of cells, given that the total number of granule cell neurons is ~240,000 in young adult C57BL/6 mice (39). The maintenance of a small fraction of Arc activity during a behavioral experience is critical for proper hippocampal function, and is consistent with electrophysiologic recordings showing sparse activity in the DG during behavior (40), and with the principle of sparse distributed coding (41). This principle suggests that the maximally efficient storage/function requires only a fraction of the total population of cells in the DG (42). Thus, a modest reduction in the number of cells expressing Arc in the hippocampal DG may be sufficient to

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**Figure 4.** Percentages of granule cell neurons expressing Arc mRNA and Arc protein after exploration of a novel environment. The data represent analyses of tissues from nonirradiated (A and C) and irradiated (B and D) mice either 1 wk (A and B) or 2 mo (C and D) after treatment. After exploration of a novel environment, the fraction of neurons expressing Arc mRNA and Arc protein were significantly higher than caged control animals except for 2 mo after irradiation. Columns, mean averages of six mice; bars, SE (*, P < 0.001).
disrupt the finely regulated sparse coding, and thereby decrease the memory capacity of the system.

A moderate dose of X-rays, as used here, did not affect the percentage of granule cell neurons that expressed behaviorally induced Arc mRNA 1 week after exposure (Fig. 5A), but was sufficient to significantly reduce the percentage of neurons expressing Arc protein (Fig. 5B). This could represent an interference of intracellular trafficking or involve the disruption of translation in the dendrites in which Arc mRNA is regulated by synaptic signals such as brain-derived neurotrophic factor and reelin (43, 44). The early effects seen here could also be due to alterations in turnover and/or translation-regulatory RNA-binding proteins (45). Alternatively, because many radiation-induced IEG effects are influenced by alterations in proteosome processing (46), our findings could represent a faster degradation of Arc protein after irradiation. Regardless of the mechanism(s) involved, there seems to be a clear disconnect between changes related to transcription and translation seen 1 week after irradiation. This finding is consistent with a previous in vitro report demonstrating that ionizing irradiation modifies gene transcription and translation activity through different mechanisms (47).

In contrast to what was seen 1 week after irradiation, there was a significant (~40%) reduction in the percentage of granule cell neurons that expressed behaviorally induced Arc mRNA 2 months after radiation treatment (Fig. 5C). The similar reduction in behaviorally induced Arc protein (Fig. 5D) was more substantial than that seen at the earlier time point (Fig. 5B). The more robust changes in the expression of Arc protein may be responsible for the corresponding changes in Arc mRNA through a complex feedback mechanism, and may suggest that the effect of irradiation on translational control may serve as a regulator of transcription (47, 48); however, this idea is speculative at this time and a more comprehensive study is required. Although the molecular mechanisms responsible for radiation-induced cognitive injury are not yet known, the 40% reductions in Arc mRNA and Arc protein seen here may be involved. For instance, recent studies have reported that Arc is involved in the trafficking of glutamate (AMPA) receptors in hippocampal neurons (49) and that AMPA receptors regulate Arc (50). Based on those data, it was proposed that changes in the NMDA/AMPA receptor ratio might enhance the negative feedback control of Arc expression (50), thus influencing the transcription of Arc. Because changes in NMDA receptors in the hippocampal formation are associated with cognitive impairment after irradiation (34), one possible mechanistic explanation is that irradiation may lead to alteration in the AMPA/NMDA receptor ratio, resulting in a reduction of Arc expression. Another explanation may involve reduced neurogenesis which we have previously shown to be associated with radiation-induced cognitive impairment (14, 15). It is particularly germane to this idea that the fraction of newly born neurons in the dentate subgranular zone expressing Arc is almost 2-fold higher than what is seen in more mature cells (51). These and/or other possibilities need to be further explored and may provide the mechanistic link between Arc expression and neuronal dysfunctions that ultimately result in altered cognitive function after brain irradiation.

The data shown here suggest that a dose of radiation known to induce cognitive impairments (14–17) initiates early changes that perturb neuronal activity without affecting neuronal survival. This may be due, in part, to radiation-induced changes in the microenvironment such as oxidative stress or neuroinflammation, which have been shown to affect cells in the hippocampus (15, 28, 31, 32, 52). In our study, we hypothesized that radiation-induced changes in the fraction of Arc-expressing cells could involve neuroinflammation, and quantified the number of activated microglia in and around the DG (Fig. 6). Although there was some indication of increased numbers of activated microglia at 1 week, this change became significant at 2 months following exposure (Fig. 6). Because the fraction of cells expressing Arc protein was significantly reduced at both time points, whereas the number of activated microglia were only significantly elevated at 2 months, this suggested that the presence of inflammatory cells per se was probably not linked to the reduction in the numbers of neurons expressing Arc protein. On the other hand, there may be an association between increased numbers of activated microglia and the later (2 months) changes in Arc mRNA; data does exist in other models of brain injury supporting a link between Arc expression and neuroinflammation (29). However, at this time, it is not known if the changes observed in Arc expression after irradiation are related to the number of activated microglia or if the coincident changes are independent.

Figure 5. The effects of irradiation on the fractions of neurons expressing Arc mRNA (A and C) and Arc protein (B and D) in mice allowed to explore a novel environment. One week after irradiation, there was no effect on Arc mRNA (A) but a significant decrease in Arc protein (B). Two months after irradiation, there were significant decreases in both the fractions of neurons expressing Arc mRNA and Arc protein (C and D) when compared with nonirradiated mice. Points, mean averages of six mice; bars, SE (*, P < 0.05).
Currently, we do not understand how ionizing radiation affects neuronal function. The present study provides novel information relevant to this topic and provides insight into new approaches to address a clinically significant problem. Although Arc expression has been a topic of considerable recent interest in the context of neural networks, memory consolidation and synaptic plasticity (for a review, see ref. 53), this is the first report of the characterization of Arc expression in a mouse model of brain irradiation. Given the current availability of mutant mouse models, it will now be possible to fully address more specific questions regarding the cascade of molecular events associated with Arc expression, and if and how those events affect the development of cognitive changes after brain irradiation. Such information is presently unavailable but is essential for determining the risks of specific central nervous system–related effects and for the development of potential strategies to manage radiation-mediated brain injury.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Figure 6. Representative images of the reconstructed DG of a mouse 2 months after irradiation with 0 Gy (A) or 10 Gy (B). Nuclei were counterstained (green); activated microglia (CD68+; red). A typical activated microglial cell (inset). Irradiation is associated with increased numbers of activated microglia. Irradiated animals exhibited a significant increase in the average number of activated microglia cell/mm² (C). Points, mean averages of six mice; bars, SE (*, P < 0.05). Bar, 100 μm.
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