

Gadolinium Retention in the Central and Peripheral Nervous System: Implications for Pain, Cognition, and Neurogenesis

Safia M. Alkhunizi, MS • Marc Fakhoury, PhD • Wassim Abou-Kheir, PhD • Nada Lawand, PhD

From the Department of Anatomy, Cell Biology and Physiological Sciences (S.M.A., M.F., W.A., N.L.) and Department of Neurology (N.L.), Faculty of Medicine, American University of Beirut, PO Box 11-0236, Riad El-Solh, Diana Tamari Sabbagh (DTS) Building, Bldg 130, John Kennedy St, Beirut 1107 2020, Lebanon. Received December 4, 2019; revision requested January 15, 2020; revision received June 10; accepted June 16. Address correspondence to N.L. (e-mail: nl08@aub.edu.lb).

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Conflicts of interest are listed at the end of this article.

See also the editorial by Radbruch in this issue.

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Background: Despite the wide use of gadolinium-based contrast agents (GBCAs) for enhanced MRI, their neurochemical and behavioral consequences, if any, remain poorly understood.

Purpose: To investigate the effect of repeated exposure to a linear or macrocyclic GBCA on gadolinium retention in the central and peripheral nervous system of rats and to assess the functional implications of such retention on hippocampal neurogenesis and sensory and cognitive processing.

Materials and Methods: Seventy male Sprague-Dawley rats (4 weeks old) received intraperitoneal injections of gadoterate meglumine (0.6 or 2.5 mmol per kilogram of body weight), gadodiamide (0.6 or 2.5 mmol/kg), or saline daily for 20 days (February 2018–March 2019). The 5-bromo-2'-deoxyuridine injections were administered every 3 days to determine the number of proliferating cells and the number of newly maturing neurons in the hippocampus. Sensory and cognitive behavioral tests were performed to assess the effect of GBCAs on pain sensitivity and spatial working memory function, respectively. Finally, inductively coupled plasma mass spectrometry analysis was used to quantify gadolinium retention in the brain, spinal cord, and peripheral nerves 24 hours after the last GBCA administration. One-way and mixed-design analyses of variance were used for statistical analysis.

Results: All GBCAs resulted in significant gadolinium retention in central and peripheral nervous tissues (1.8–333.2 nmol Gd/g tissue). Pain hypersensitivity to thermal and mechanical stimuli ($P < .001$) was observed after gadodiamide exposure in rats but not after gadoterate meglumine exposure. Rats injected with both GBCAs showed no changes in spatial working memory or in hippocampal cell proliferation and maturation.

Conclusion: Gadolinium was retained in the spinal cord and peripheral nerves in rats exposed to multiple administrations of linear and macrocyclic contrast agents. Gadodiamide (linear contrast agent) but not gadoterate meglumine (macrocyclic contrast agent) led to pain hypersensitivity, but neither affected spatial working memory performance, hippocampal cellular proliferation, or hippocampal neurogenesis.

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Gadolinium-based contrast agents (GBCAs) are chemical substances used in conjunction with MRI to visualize internal organs and to diagnose and monitor disease progress. After their introduction in 1982 at the annual meeting of the Radiological Society of North America, GBCAs have transformed and revolutionized diagnostic medical imaging (1). In 1988, the Food and Drug Administration approved the first GBCA for clinical use, and currently nine agents are available for commercial use (2,3). In 2014, however, Kanda et al (4) reported the clinical observation of an increased signal intensity in the dentate nucleus and globus pallidus at unenhanced MRI in patients with previous exposure to GBCAs, indicating for the first time gadolinium retention in the brain. Despite the wide use of GBCAs, more research is needed to investigate whether they result in any physiologic or behavioral impairment.

On the basis of their molecular structure, GBCAs can be classified as linear or macrocyclic; according to their global charge, they can be classified as ionic or nonionic (5). Linear GBCAs are characterized by an open chair elongated linear structure of the ligand, whereas macrocyclic agents are characterized by a cage-like structure and exhibit higher thermodynamic and kinetic stability (5). Both types of GBCAs can lead to gadolinium retention in the brain, although retention is higher after treatment with the linear form (6–9). Gadolinium retention after exposure to GBCAs is also observed in areas outside the brain, including the skin (10), bone (11,12), and liver (8). However, the functional implication of such metal retention on hippocampal neurogenesis, the most drastic form of brain plasticity in adulthood, and other neural processes (eg, pain sensitivity and working memory performance) remains to be elucidated.

Abbreviations

ANOVA = analysis of variance, BrdU = 5-bromo-2'-deoxyuridine, GBCA = gadolinium-based contrast agent, ICP-MS = inductively coupled plasma mass spectrometry, NeuN = hexaribonucleotide binding protein-3

Summary

Contrast agents administered daily led to gadolinium retention in rat central and peripheral nervous tissues; gadodiamide but not gadoterate meglumine led to pain hypersensitivity, but neither altered cognitive function or neurogenesis.

Key Results

- Repeated injections of gadodiamide (linear) and gadoterate meglumine (macrocytic) in healthy rats at 0.6 or 2.5 mmol per kilogram of body weight per day for 20 days led to gadolinium (Gd) retention in the cerebrum, spinal cord, and peripheral nerves (range, 1.8–333.2 nmol Gd/g tissue).
- Gadodiamide but not gadoterate meglumine led to pain hypersensitivity (latency of paw withdrawal to radiant heat, up to 4 seconds \pm 0.2 [standard error]; $P < .05$).
- Neither type of contrast agent affected spatial working memory performance, hippocampal cellular proliferation, or hippocampal neurogenesis.

The purpose of this study is fourfold: First, to investigate whether exposure to a linear or macrocyclic GBCA leads to gadolinium retention in the central and peripheral nervous tissues; second, to assess the effect of these GBCAs on sensory behavior; third, to assess whether GBCA exposure affects cognitive functions (eg, working memory performance); and fourth, to investigate whether multiple exposures to a linear or macrocyclic GBCA in rats affects cellular proliferation and neurogenesis in the hippocampus.

Materials and Methods

Animals

Seventy male Sprague-Dawley rats (4 weeks old) weighing 140–150 g at the start of the experiments served as subjects. Animals were housed with a controlled temperature range (20–22°C) and a 12-hour light-dark cycle and were provided with water and food ad libitum. All experimental procedures were conducted in accordance with the ethical guidelines and under the approval of the institutional animal care and use committee at the American University of Beirut.

Treatment Regimen

Rats were divided into five experimental groups (six rats per group): (a) group 1 received intraperitoneal injections of gadodiamide, a linear nonionic GBCA (Omniscan; GE Healthcare, Munich, Germany) at a high dose of 2.5 mmol/kg; (b) group 2 received intraperitoneal injections of gadodiamide at a low dose of 0.6 mmol/kg; (c) group 3 received intraperitoneal injections of gadoterate meglumine, a macrocyclic ionic GBCA (Dotarem; Guerbet, Villepinte, France) at a high dose of 2.5 mmol/kg; (d) group 4 received intraperitoneal injections of gadoterate meglumine at a low

dose of 0.6 mmol/kg; and (e) group 5 received intraperitoneal injections of the vehicle (0.9% saline) and served as a control. The GBCAs and saline were injected daily during a period of 20 days (Fig 1, A). The low dose is equivalent to the clinically administered human dose (13), and the high dose represents the optimal dose required for brain MRI enhancement after intraperitoneal administration of GBCA in rodents (14).

Heat Hyperalgesia Test

Heat hyperalgesia was assessed in the different experimental groups (six rats per group). The test was conducted by measuring foot withdrawal latency to radiant heat stimulus (intensity of 40 infrared units) applied to the plantar surface of the hind paw. Approximately 30 minutes before testing, rats were put individually into a clear plastic cage for accommodation. Heat stimuli were applied three times, with a 10-minute rest period between trials. Testing was performed before GBCA injection and at days 7, 14, and 20 after injection of tested GBCAs (Fig 1, A). Withdrawal latency was defined as the elapsed time, in seconds, from stimulus onset to paw withdrawal. Tests were performed by one author (S.M.A., 3 years of experience).

Mechanical Hyperalgesia Test

Frequency of paw withdrawal after application of a von Frey filament with a bending force of 15 g to the plantar surface of the hind paw was assessed in rats treated with the low (0.6 mmol/kg) and high (2.5 mmol/kg) doses of both GBCAs (six rats per group). Testing started by poking the plantar surface five successive times separated by a 5-minute interval. The paw withdrawal frequency was reported and defined as the number of paw withdrawals per trial. The mean of the five trials was considered. Testing was performed by one author (S.M.A.) before GBCA injection (for baseline values) and at days 7, 14, and 20 after GBCA injection (Fig 1, A).

Animal Euthanasia and Perfusion

Rats were euthanized 24 hours after the last GBCA injection (day 21) (Fig 1, A). They were deeply anesthetized via intraperitoneal injection of ketamine (80 mg/kg, Ketalar; Panpharma, Luitré, France) and xylazine (10 mg/kg, Xyla; Interchemie, Harju County, Estonia). They were then cardially perfused with 200 mL of 0.9% normal saline for blood displacement followed by 4% formalin solution for tissue fixation and finally euthanized via decapitation.

Inductively Coupled Plasma Mass Spectrometry Analysis for Gadolinium Tissue Detection and Quantification

For gadolinium tissue detection in the nervous system 24 hours after the last GBCA injection (day 21) (Fig 1, A), the cerebrum (four rats per group), whole spinal cords (five rats per group), and peripheral nerves (sciatic and trigeminal nerves, four rats per group) were extracted and chemically digested at approximately 180°C for 30 minutes (15). With each digested batch of samples, a blank, a spiked

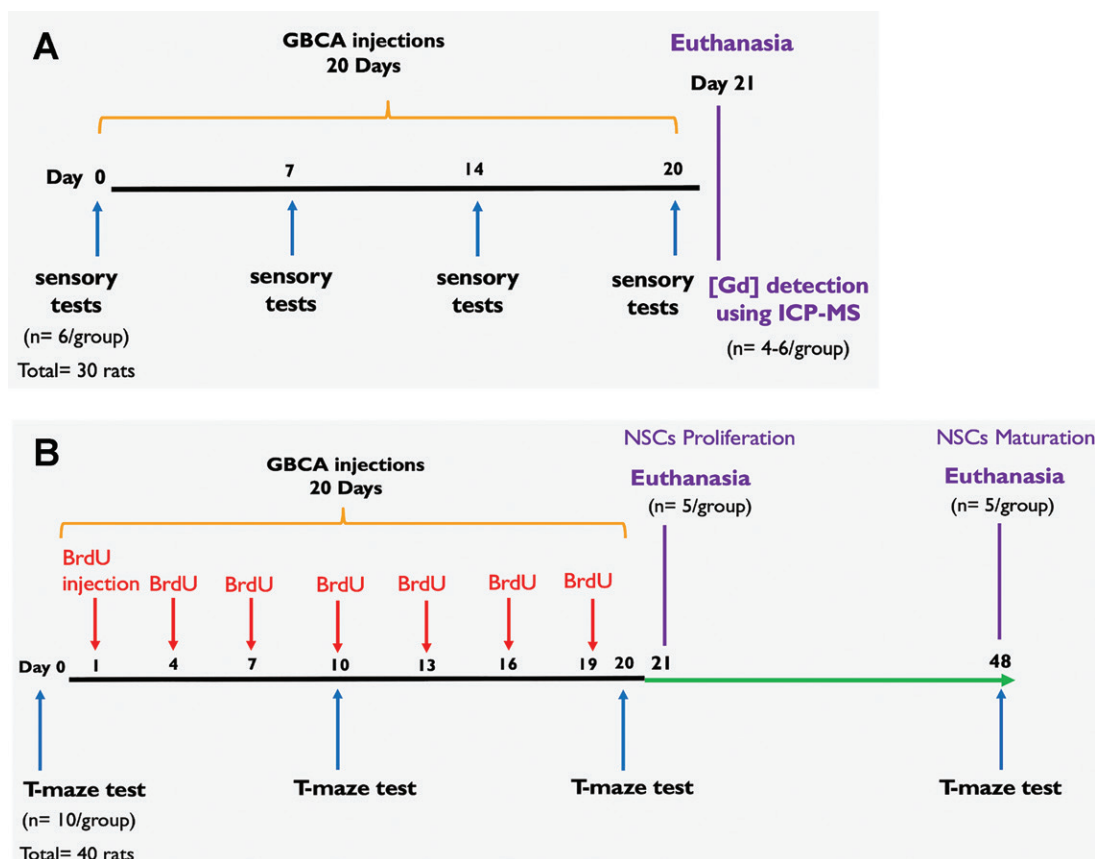


Figure 1: Experimental design. Two experiments were conducted in the study. *A*, The experimental timeline of gadolinium-based contrast agent (GBCA) injections (20 consecutive days), heat and mechanical hyperalgesia tests (days 0, 7, 14, and 20), euthanasia time point, and the inductively coupled plasma mass spectrometry (ICP-MS) analysis for gadolinium (Gd) tissue quantification (day 21). In total, 30 rats were used in experiment 1. *B*, The experimental timeline of GBCA injections (20 consecutive days), 5-bromo-2'-deoxyuridine (BrdU) injections (every 3 days), spontaneous alternation T-maze test (days 0, 10, 20, and 48), immunofluorescence analysis for hippocampal neural stem cell (NSC) proliferation (day 21) and maturation (day 48), and euthanasia time points (days 21 and 48). In total, 40 rats were used in experiment 2. The number of animals used in each experiment is indicated in the diagram.

blank, a certified reference material sample, and a matrix spike were used as quality control. The diluted samples and quality control were measured by using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce; Agilent, Waldbronn, Germany). All gadolinium quantifications were blindly performed by an analytical chemist with more than 5 years of experience.

Hippocampal Neurogenesis: Bromodeoxyuridine Administration

To assess the effect of intracranial gadolinium retention on hippocampal neurogenesis and spatial working memory, another set of 4-week-old male Sprague-Dawley rats (140–150 g) was used (Fig 1, *B*). Rats were divided into four experimental groups (10 rats per group): (*a*) group 1 received gadodiamide (2.5 mmol/kg), (*b*) group 2 received gadoterate meglumine (2.5 mmol/kg), (*c*) group 3 received saline, and (*d*) group 4 consisted of treatment-naïve rats. GBCAs and saline were injected intraperitoneally every day during a 20-day period, and all experimental groups received intraperitoneal injections of 5-bromo-2'-deoxyuridine (BrdU) (300 μ L, 48.6 mg/kg per injection; Sigma-Aldrich, St Louis, Mo) every 3 days throughout the 20-day period (Fig 1, *B*).

Spontaneous Alternation T-Maze Test

The spontaneous alternation T-maze test was performed in rats treated with the high dose of both GBCAs (2.5 mmol/kg) before treatment, at days 10 and 20 during the GBCA exposure period ($n = 10$ /group), and 28 days after the last GBCA injection (day 48) (five rats per group) (Fig 1, *B*) (16). Briefly, after 15 minutes of habituation, rats were placed at the starting arm of the T-maze and could choose between the right and left goal arms. After entry, a barrier was used to entrap the animal for 30 seconds, then the rat was placed back at the original position and allowed to choose between the open arms. Three trials at each time point were performed by a trained experimenter (S.M.A.), and the percentages of the successful trials were compared among the different groups.

Brain Sectioning: Collection of the Dentate Gyrus of the Hippocampus

Rats were euthanized via cardiac perfusion on days 21 (five rats per group) and 48 (five rats per group) of the experiment for the assessment of cellular proliferation and neuronal maturation in the dentate gyrus of the hippocampus, respectively (Fig 1, *B*). Brains were extracted and postfixed in 4% paraformaldehyde solution overnight then transferred to 30% sucrose in

0.1 mmol/L of phosphate-buffered saline solution before being stored at 4°C. The entire dentate gyrus region of the hippocampal formation was collected and divided into the rostral, intermediate, and caudal region, with coordinates taken from the rat brain atlas (17). Coronal tissue sections from each of the three regions of the hippocampus were collected following the fractionator principle (18,19).

Immunofluorescence

Immunofluorescence staining was performed as described earlier (19). Free-floating hippocampal brain sections were washed with 0.1 mmol/L phosphate-buffered saline followed by an incubation with 2N hydrochloric acid for 30 minutes at 37°C for antigen retrieval. Tissue sections were then incubated with the primary antibody rat monoclonal anti-BrdU (1:100) (Bio-Rad, Hertfordshire, England) and kept overnight at 4°C. On the 2nd day, tissues were incubated in the dark with the secondary antibody, Alexa Fluor-568 goat anti-rat (Invitrogen; ThermoFisher Scientific, Eugene, Ore), for 2 hours at room temperature. Tissue sections were then incubated with the mouse monoclonal anti-hexaribonucleotide binding protein-3 (NeuN) (1:400) (Millipore, Darmstadt, Germany) overnight at 4°C. On the 3rd day, sections were incubated with the secondary antibody Alexa Fluor-488 goat antimouse (1:250) (Invitrogen; ThermoFisher Scientific) for 2 hours at room temperature. Finally, Hoechst stain (1:10 000) (Invitrogen, ThermoFisher Scientific) was applied to the sections for 10 minutes and hippocampal tissues were mounted on glass slides.

Confocal Microscopy and Cell Quantification

The number of BrdU⁺ and BrdU⁺/NeuN⁺ cells in the dentate gyrus of the hippocampus was examined with laser-scanning confocal microscopy (Zeiss LSM 710; Zeiss, Oberkochen, Germany) under a 40× oil objective to quantify the number of proliferating cells and newly maturing neurons, respectively. Confocal images were analyzed by using the ZEN (Zeiss) image analysis software. All slides were blindly reviewed (N.L., W.A.; >15 years of experience).

Statistical Analysis

For the BrdU cell count and ICP-MS results, one-way analysis of variance (ANOVA) was performed, followed by the Tukey test for post hoc analysis. For sensory tests, a mixed-design ANOVA was conducted to account for the change of hyperalgesia response among different treatments across time. One-way ANOVA was used to detect hyperalgesia across different treatments, with the Dunnett test for post hoc analysis. Multiple paired *t* tests with baseline time as a reference were then performed within each treatment group. For the T-maze test, the alteration rate was recorded, data were normalized, and a mixed-design ANOVA was conducted. Effect size was measured and reported as partial eta squared (η_p^2) values to denote small ($\eta_p^2 > 0.01$), medium ($\eta_p^2 > 0.06$), or large ($\eta_p^2 > 0.14$) effect sizes (20). Data are expressed as mean \pm standard error of the mean. All statistical analyses were performed (S.M.A., M.F.; 3 and 8 years of experience, respec-

tively) by using IBM SPSS Statistics, version 24 (IBM, Armonk, NY); a *P* value less than .05 was considered to indicate a significant difference.

Results

Determination of Gadolinium Concentration in the Central and Peripheral Nervous System Using ICP-MS

Both tested GBCAs resulted in gadolinium retention in the cerebrum (Fig 2, A), spinal cord (Fig 2, B), and peripheral nerves (Fig 2, C) (Table). In rats treated with the low dose (0.6 mmol/kg) of both GBCAs, the mean detected gadolinium tissue concentrations were significantly higher than those detected in the control group in the cerebrum (saline, 0.06 ± 0.02 ; gadodiamide, $5.2 \text{ nmol/g} \pm 1.4$ [$P = .01$]; gadoterate meglumine, $1.8 \text{ nmol/g} \pm 0.3$ [$P = .001$]), spinal cord (saline, 0.3 ± 0.2 ; gadodiamide, $13.2 \text{ nmol/g} \pm 2.6$ [$P = .001$]; gadoterate meglumine, $1.9 \text{ nmol/g} \pm 0.4$ [$P = .005$]), and peripheral nerves (saline, 0.3 ± 0.1 ; gadodiamide, $91.7 \text{ nmol/g} \pm 25.9$ [$P = .01$]) except for gadoterate meglumine treatment in the peripheral nerves ($0.6 \text{ nmol/g} \pm 0.2$; $P = .32$). In the spinal cord, the mean detected residual gadolinium concentration after exposure to gadodiamide (0.6 mmol/kg) was approximately sevenfold higher compared with gadoterate meglumine exposure at the same dose ($13.2 \text{ nmol/g} \pm 2.6$ vs $1.9 \text{ nmol/g} \pm 0.4$, $P = .003$) (Fig 2, B).

After treatment with the high dose (2.5 mmol/kg), the mean detected residual gadolinium concentration in the spinal cord after exposure to gadodiamide was approximately 9.4-fold higher compared with gadoterate meglumine exposure ($47.6 \text{ nmol/g} \pm 6.7$ vs $5.0 \text{ nmol/g} \pm 0.6$, $P < .001$) (Fig 2, B). In addition, the mean detected total gadolinium concentrations were significantly higher than those detected in the control group in the cerebrum (saline, 0.06 ± 0.02 ; gadodiamide, $8.6 \text{ nmol/g} \pm 0.5$ [$P < .001$]; gadoterate meglumine, $8.3 \text{ nmol/g} \pm 1.0$ [$P < .001$]), spinal cord (saline, 0.3 ± 0.2 ; gadodiamide, $47.6 \text{ nmol/g} \pm 6.7$ [$P < .001$]; gadoterate meglumine, $5.0 \text{ nmol/g} \pm 0.6$ [$P < .001$]), and peripheral nerves (saline, 0.3 ± 0.1 ; gadodiamide, $333.2 \text{ nmol/g} \pm 82.0$ [$P = .007$]; gadoterate meglumine, $3.2 \text{ nmol/g} \pm 1.0$ [$P = .03$]).

Effect of GBCA Exposure on Heat and Mechanical Hyperalgesia

Exposure to gadodiamide at the high dose induced heat hyperalgesia at day 14 in the right paw (withdrawal latency, $4.7 \text{ seconds} \pm 0.3$) and left paw ($4.7 \text{ seconds} \pm 0.4$) as compared with baseline (right paw, $7.7 \text{ seconds} \pm 0.6$ [$P = .007$]; left paw, $7.5 \text{ seconds} \pm 0.8$ [$P = .049$]) and the control group (right paw, $7.2 \text{ seconds} \pm 0.5$ [$P = .003$]; left paw, $8.2 \text{ seconds} \pm 0.8$ [$P < .001$]). A similar trend was observed at day 20 for both doses of gadodiamide (Fig 3, A). There was a significant treatment by time interaction for the right and left paws ($P < .001$, $\eta_p^2 = 0.53$; $P = .047$, $\eta_p^2 = 0.35$, respectively).

Similarly, exposure to gadodiamide (2.5 mmol/kg) resulted in mechanical hyperalgesia at day 20 in the right paw (withdrawal frequency, $3.9 \text{ withdrawals per trial} \pm 0.2$; $P =$

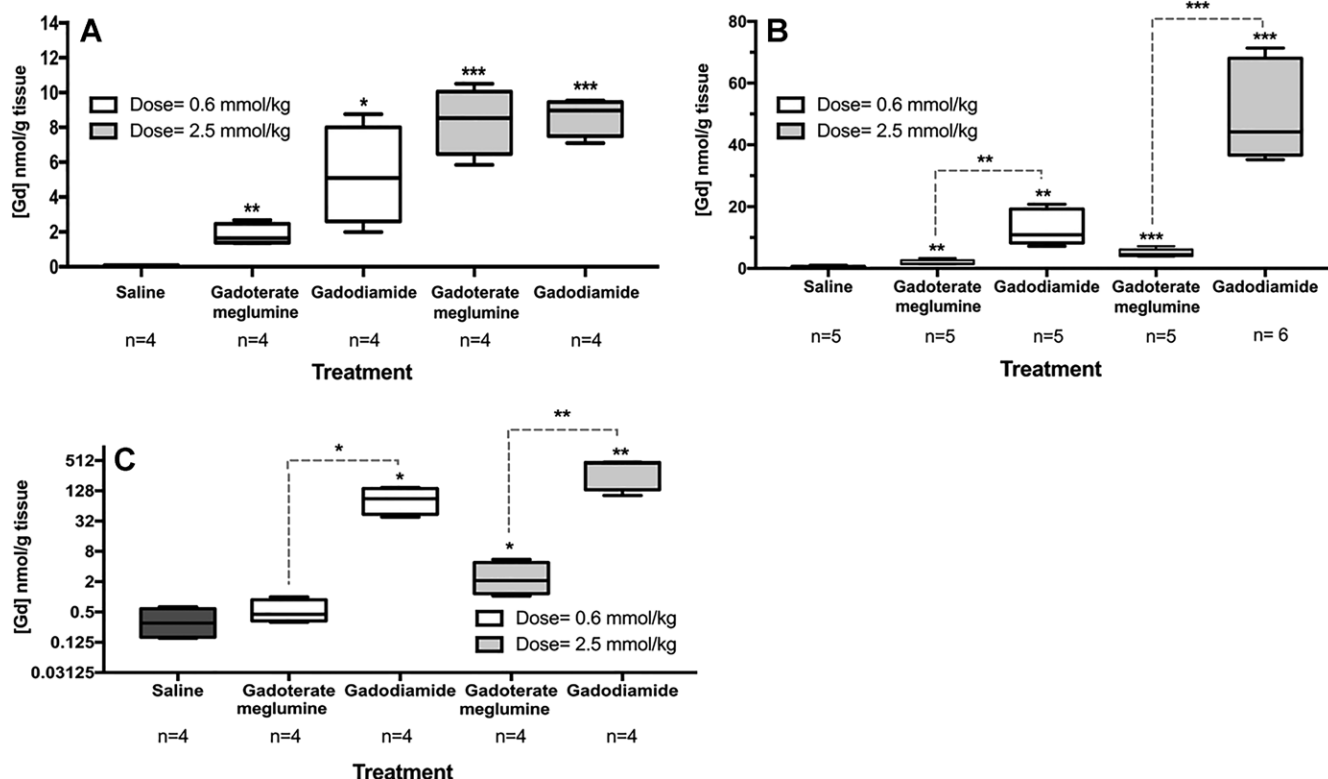


Figure 2: Effect of gadolinium-based contrast agent (GBCA) exposure (0.6 mmol/kg and 2.5 mmol/kg) on gadolinium (Gd) retention in the central and peripheral nervous system. Findings shown for, A, the cerebrum area extending from the optic chiasm to the midbrain (region covering the hippocampal formation); B, the spinal cord; and, C, the peripheral nerves (sciatic and trigeminal nerves). In all analyzed regions at 24 hours after the last GBCA injection (day 21), gadodiamide exposure resulted in higher gadolinium tissue concentration than gadoterate meglumine exposure. The concentration of gadolinium is expressed in nanomoles per gram of tissue. The limit of quantification was 0.013 nmol of gadolinium per gram of wet tissue. In the graphs, boundaries of boxes represent the interquartile range, and the lines inside boxes represent the median. The number of animals tested in the different groups is indicated below the graph. Statistical significance with reference to the control group or between the gadoterate meglumine- and gadodiamide-treated groups (dashed arrows) is indicated. * = $P < .05$, ** = $P < .01$, and *** = $P < .001$.

Results of Inductively Coupled Plasma Mass Spectrometry: Amount of Gadolinium in Tissue

Group	Cerebrum* ($n = 4$ /group) (nmol/g)	Spinal Cord ($n = 5$ /group) (nmol/g)	Peripheral nerves† ($n = 4$ /group) (nmol/g)
Control (saline) group	0.06 ± 0.02	0.3 ± 0.2	0.3 ± 0.1
Gadoterate meglumine group			
0.6 mmol/kg dose	1.8 ± 0.3	1.9 ± 0.4	0.6 ± 0.2
2.5 mmol/kg dose	8.3 ± 1.0	5.0 ± 0.6	3.2 ± 1.0
Gadodiamide group			
0.6 mmol/kg dose	5.2 ± 1.4	13.2 ± 2.6	91.7 ± 25.9
2.5 mmol/kg dose	8.6 ± 0.5	47.6 ± 6.7	333.2 ± 82.0

Note.—Data are mean \pm standard error of the mean. Gadolinium tissue quantification using inductively coupled plasma mass spectrometry in the cerebrum, spinal cord, and peripheral nerves of rats treated with 20 intraperitoneal injections of gadodiamide and gadoterate meglumine.

* Cerebrum refers to the cerebral area extending from the optic chiasm to the midbrain.

† Peripheral nerves included the right and left sciatic nerves, in addition to the trigeminal nerves.

.004 vs control and $P = .01$ vs baseline) and left paw (withdrawal frequency, 4.1 withdrawals per trial \pm 0.3; $P < .001$ vs control and $P < .001$ vs baseline) (Fig 3, B). Mechanical hyperalgesia was also observed at day 14 in rats treated with the high dose of gadodiamide. There was a significant treatment by time interaction for the right and left paws ($P < .001$, $\eta_p^2 = 0.77$; $P < .001$, $\eta_p^2 = 0.76$, respectively).

Effect of GBCA Exposure on Spatial Working Memory

The effect of gadodiamide and gadoterate meglumine (2.5 mmol/kg) exposure on spatial working memory was assessed with the spontaneous alternation T-maze test at days 10, 20, and 48. Cognitive performance was not significantly affected across time ($P = .31$, $\eta_p^2 = 0.07$) or among the different treatments ($P = .51$, $\eta_p^2 = 0.13$) (Fig 4). Thus, exposure to dif-

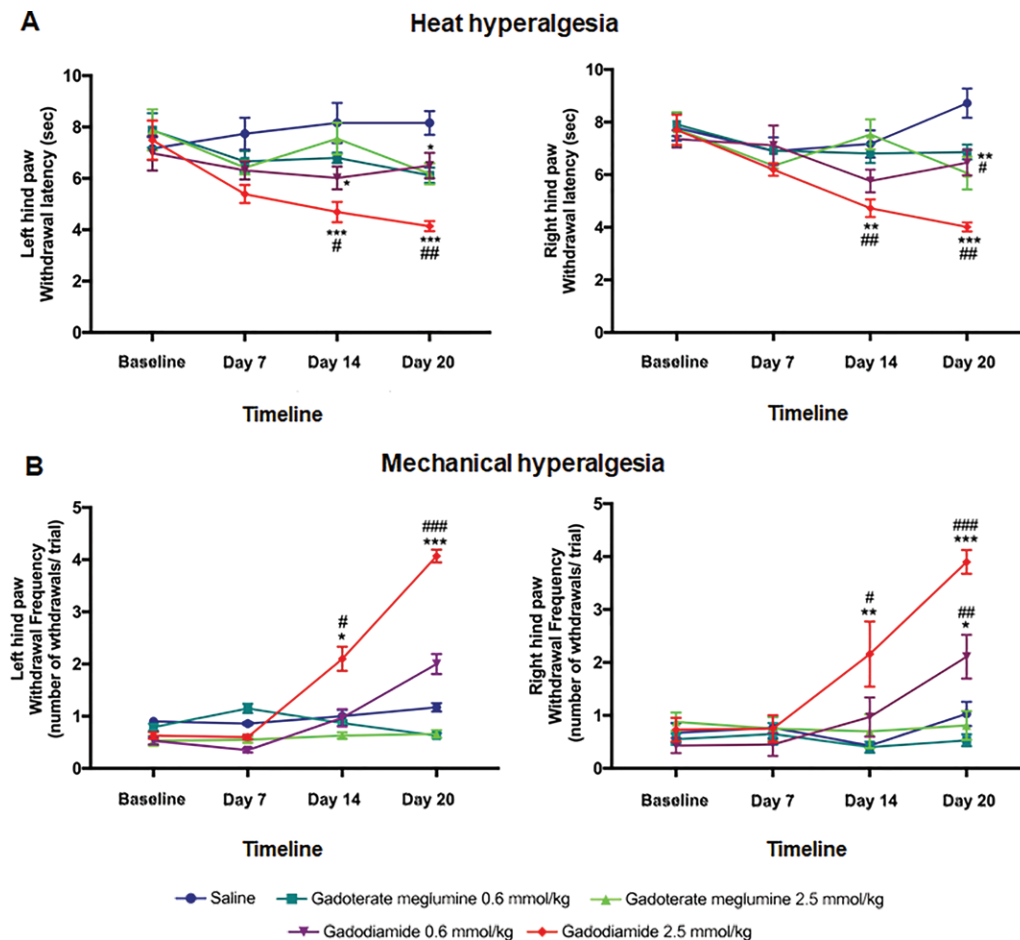


Figure 3: Effect of gadolinium-based contrast agent (GBCA) exposure (0.6 and 2.5 mmol/kg) on heat and mechanical hyperalgesia. A, Time course of heat hyperalgesia in the left (left panel) and right (right panel) hind paw of rats treated with GBCAs (0.6 and 2.5 mmol/kg) or saline (control) on days 7, 14, and 20 of the GBCA exposure period. B, Time course of mechanical hyperalgesia (number of withdrawals per trial) in the left (left panel) and right (right panel) hind paw of rats treated with GBCAs (0.6 and 2.5 mmol/kg) or saline (control). Data are expressed as mean \pm standard error of the mean (six rats per group). Statistical significance between the experimental groups and the control group was assessed by using one-way analysis of variance followed by two-tailed Dunnett test, with rats that received saline serving as the reference group. * = $P < .05$, ** = $P < .01$, *** = $P < .001$. Statistical significance within groups across time points was evaluated with the multiple paired t test, with baseline time as the reference group. # = $P < .05$, ## = $P < .01$, ### = $P < .001$.

ferent GBCAs did not affect the alternation rate or spatial working memory across time ($P = .34$, $\eta_p^2 = 0.19$).

Effect of GBCA Exposure on Cellular Proliferation and Neurogenesis in the Dentate Gyrus of the Hippocampus

The effect of GBCA exposure on cellular proliferation and neurogenesis in the dentate gyrus of the hippocampus was assessed by counting the number of BrdU⁺ cells on day 21 and the number of BrdU⁺/NeuN⁺ colabeled cells on day 48 (Figs 5, 6). Administration of GBCAs (2.5 mmol/kg) for 20 consecutive days failed to induce alterations in cellular proliferation (gadodiamide, 29 395 BrdU⁺ cells \pm 4324; gadoterate meglumine, 35 830 BrdU⁺ cells \pm 3901; saline, 32 152 BrdU⁺ cells \pm 5698 [$P = .64$]) (Fig 5, C). Representative confocal images of the caudal region of the hippocampal dentate gyrus are shown in Figure 5, A and B. Administration of both GBCAs also failed to alter the neuronal differentiation process, maturation, and integration into the granule neuro-

nal layer of the dentate gyrus (gadodiamide, 28 260 BrdU⁺/NeuN⁺ cells \pm 3697; gadoterate meglumine, 36 798 BrdU⁺/NeuN⁺ cells \pm 3121; saline, 36 425 BrdU⁺/NeuN⁺ cells \pm 3214 [$P = .13$]) (Fig 6, C). Representative confocal images of the newly born neurons in the dentate gyrus of the hippocampus are shown in Figure 6, A and B.

Discussion

The present study assessed the neurochemical and behavioral effects of a linear and macrocyclic gadolinium-based contrast agent (GBCA) in rats. Our results indicate that exposure to repeated doses of a linear or macrocyclic GBCA does not alter the dynamic process of hippocampal neurogenesis or spatial working memory performance. However, our results show that repeated administration of gadodiamide but not gadoterate meglumine leads to heat and mechanical hyperalgesia in rats, suggesting that the linear GBCA but not the macrocyclic GBCA might have triggered the sensitization of spinal cord

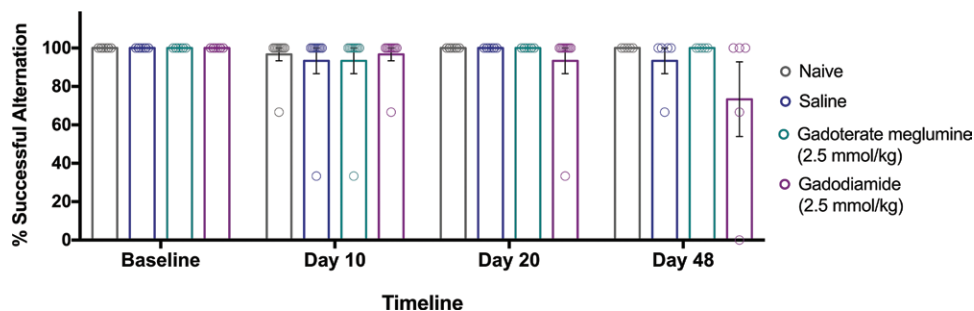


Figure 4: Effect of gadolinium-based contrast agent (GBCA) exposure (2.5 mmol/kg) on spatial working memory. Percentage of successful trials on the spontaneous alternation T-maze test in rats treated with gadoterate meglumine (2.5 mmol/kg) or gadodiamide (2.5 mmol/kg) on days 10 and 20 of the GBCA exposure period (10 rats per group) and 28 days after the last GBCA injection (day 48) (five rats per group). Data are expressed as mean \pm standard error of the mean. Individual points represent the mean of three T-maze test trials conducted on an animal. Statistical significance between treatment groups and across different time points was assessed by using mixed-model analysis of variance. Differences between experimental groups did not reach statistical significance.

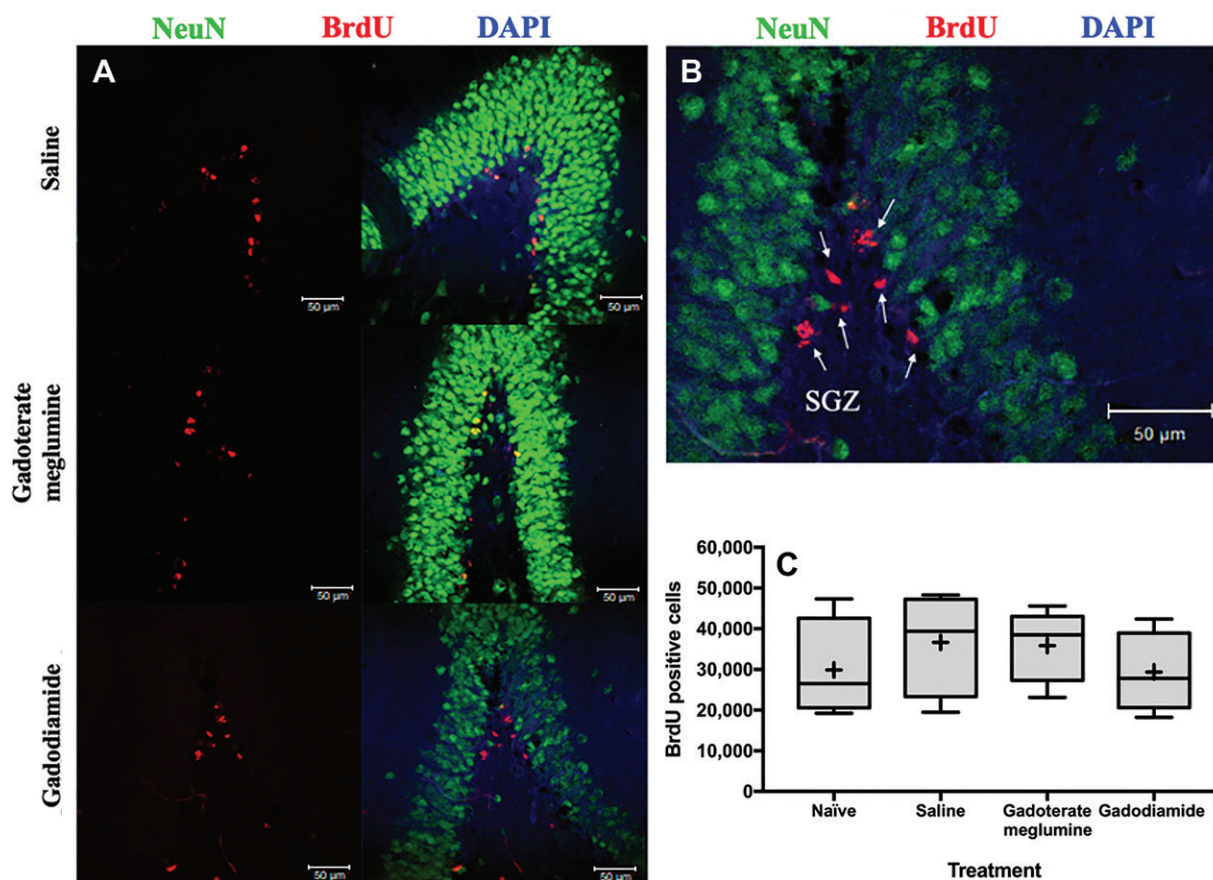


Figure 5: Effect of gadolinium-based contrast agent (GBCA) exposure (2.5 mmol/kg) on cellular proliferation in the dentate gyrus of the hippocampus. A, Representative confocal images show proliferating 5-bromo-2'-deoxyuridine (BrdU)-positive cells (red), the mature neuronal marker (NeuN; green), and the Hoechst nuclear 4',6-diamidino-2-phenylindole (DAPI) stain (blue) in the caudal region of the hippocampal dentate gyrus in experimental and control (saline-treated) groups euthanized 48 hours after the last BrdU injection (day 21). Tile scan images were obtained with a 40 \times oil objective. Scale bar, 50 μ m. B, Zoomed confocal image shows the spatial distribution of stem or progenitor BrdU-positive cells in the subgranular zone (SGZ). Tile scan images were obtained with a 40 \times oil objective. Scale bar, 50 μ m. C, Box-and-whisker plot shows quantification of the total number of proliferating BrdU-positive cells in the dentate gyrus of the hippocampus in rats exposed to 20 doses of gadodiamide and gadoterate meglumine (2.5 mmol/kg) and euthanized 48 hours after the last BrdU injection (day 21). Boundaries of boxes represent the interquartile range, the lines inside boxes represent the median, and the plus signs represent the mean values (five rats per group).

nociceptive neurons. More importantly, our results show that repeated administration of a linear GBCA and a macrocyclic GBCA (0.6 and 2.5 mmol/kg per day over 20 days) leads

to gadolinium retention in the cerebrum, spinal cord, and peripheral nerves of rats (up to 333.2 nmol/g). To our knowledge, this is the first study to provide evidence for gadolinium

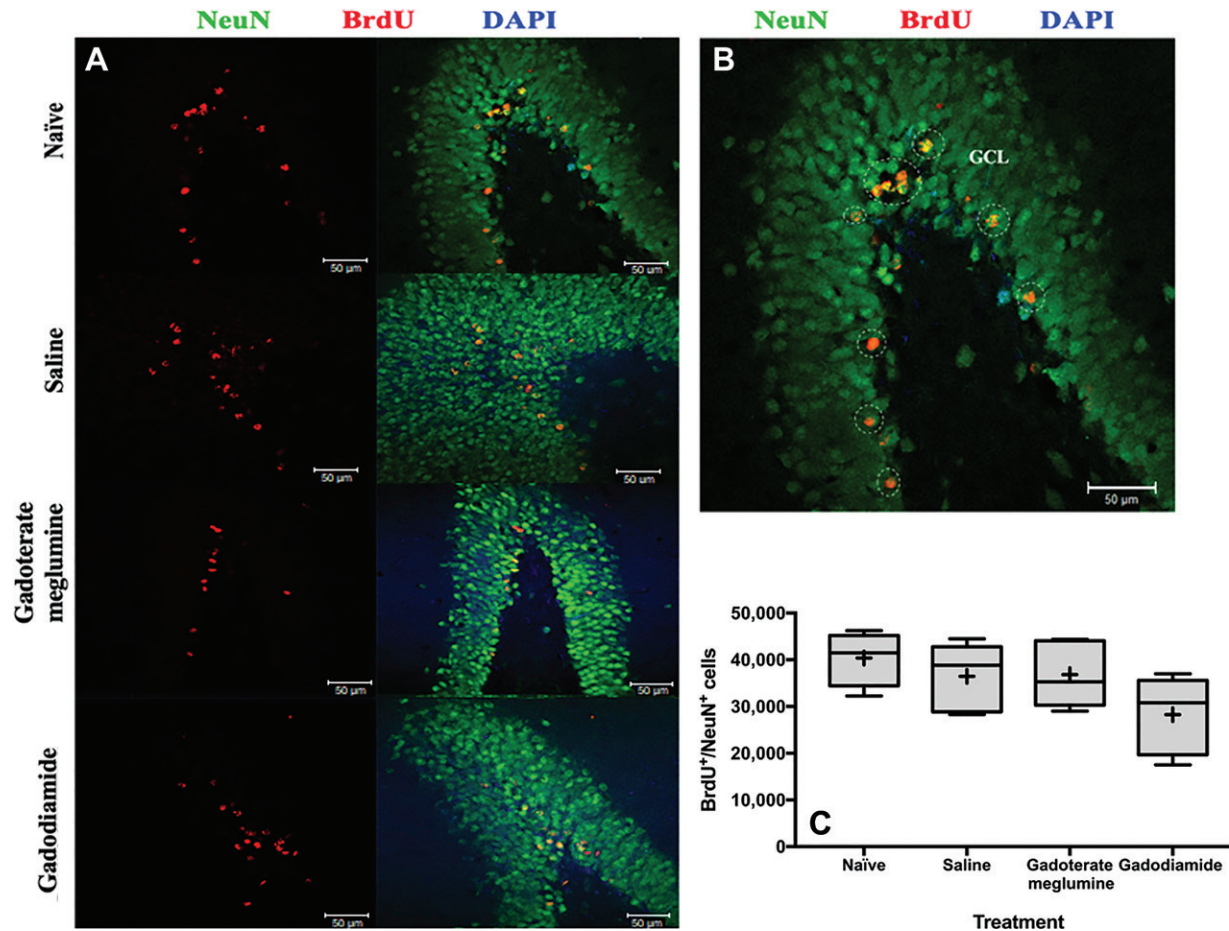


Figure 6: Effect of gadolinium-based contrast agent (GBCA) exposure (2.5 mmol/kg) on hippocampal neurogenesis. **A**, Confocal images show 5-bromo-2'-deoxyuridine (BrdU)-positive or hexaribonucleotide binding protein-3 (NeuN)-positive colabeled cells in the dentate gyrus of the hippocampus in experimental and control groups euthanized 29 days after the last BrdU injection (day 48). Newly born neurons can be seen integrated into the granular cell layer (GCL) of the dentate gyrus of the hippocampus. Tile scan images obtained with a 40 \times oil objective. Scale bar, 50 μ m. **B**, Zoomed confocal image shows the integration of newly born neurons into the granular cell layer of the dentate gyrus of the hippocampus 29 days after the last BrdU injection (day 48). Tile scan images obtained with a 40 \times oil objective. Scale bar, 50 μ m. **C**, Quantification of the total number of double-labeled BrdU-positive or NeuN-positive newly maturing neurons in the dentate gyrus of the hippocampus in rats exposed to 20 doses of gadodiamide and gadoterate meglumine (2.5 mmol/kg) and euthanized 29 days after the last BrdU injection (day 48). The total cell count represented is the summation of the number of cells positive for BrdU, NeuN, or 4',6'-diamidino-2-phenylindole (DAPI) cells counted in the rostral, intermediate, and caudal topographic regions of the hippocampus. Boundaries of boxes represent the interquartile range, lines inside boxes represent the median, and the plus signs represent the mean values (five rats per group).

retention in the spinal cord and peripheral nerves after exposure to gadodiamide and gadoterate meglumine. Gadolinium tissue concentrations detected in the cerebrum are consistent with findings from previous studies showing that the linear GBCA leads to higher gadolinium retention than the macrocyclic agent (7,8,21,22).

The high levels of brain gadolinium concentration observed in our study might be due to several factors, including the use of the intraperitoneal route of injection; this may have resulted in higher tissue retention, as suggested in previous studies (23,24). We chose the intraperitoneal route of administration in rats to avoid inducing daily stress, pain, or anesthesia exposure, which are all considered major confounding variables capable of affecting the process of hippocampal neurogenesis and cognitive behavioral outcomes. Moreover, previous animal studies followed a regimen of four or five GBCA injections per week (7,22). However, in our study, daily consecutive

injections of GBCA were performed for 20 days with no interruption, which may have contributed to our higher inductively coupled plasma mass spectrometry measurement.

The lack of significant effects of GBCAs on hippocampal neurogenesis and cellular proliferation after intracranial gadolinium retention in the developing brain of young rats is inconsistent with the clinical report by Miller et al describing a case of a pediatric patient who received a total of 35 linear GBCA injections between 8 and 20 years of age; in this patient, signal hyperintensity was evident in various brain regions after the second injection of GBCA (25). However, no definite strong association between gadolinium intracranial retention and memory dysfunction was observed in the reported case (25), which is in line with findings of our study. Moreover, our results are consistent with, and further support, the recent population-based clinical study by McDonald et al, which showed no association between GBCA exposure and neurocognitive dysfunction (26).

Our study had some limitations. A relatively small number of animals was included, which may have explained the lack of significant effect of GBCA exposure on hippocampal neurogenesis at the late time point. Moreover, our study did not include additional histologic evaluations of the spinal cord and peripheral nerves to localize the deposits and investigate potential deleterious effects of gadolinium retention. In addition, our study only examined gadolinium retention 24 hours after the last GBCA exposure. Previous studies have shown that residual gadolinium in tissues can get partially cleared over 20 weeks after injection (27,28). As such, assessing long-term gadolinium deposition would be essential for clinical relevance. Finally, even though the present work is a preclinical study that did not include human participants, the low dose (0.6 mmol/kg) that was administered in rats is equivalent to the clinically administered human dose of 0.1 mmol/kg (after adjusting for species differences and body surface area), as recommended by the Food and Drug Administration (13), and could therefore be relevant for clinical practice. In addition, given that the elimination half-life of GBCAs is approximately five times shorter in rats than in humans (22,29), the present study used multiple serial injections to induce gadolinium deposits in the rodent brain and to replicate to some extent the brain MRI signal intensity changes that are observed in humans clinically, as shown in previous animal studies (7,22,30). Nonetheless, the dosing schedule and the total administered dose in rats limit applicability of the findings of this preclinical study to humans.

Overall, our findings shed light on the effects of gadolinium-based contrast agent (GBCA) administration by revealing a significant amount of retained gadolinium at 24 hours after dosing in the spinal cord and peripheral nerves. Retention of gadolinium in the spinal cord and peripheral nerves might contribute to sensory symptoms and burning pain in the torso and extremities described by some patients after GBCA administration (31–35). Our results are of key importance for individuals who are hypersensitive to pain and who need to undergo multiple MRI examinations. Future research should focus on investigating the safety of GBCA administration and the effect of gadolinium retention on sensory and motor neuronal activities. Eventually, attention must be drawn to the long-term effect of such metal retention in the central and peripheral nervous system, especially in children and adults with medical conditions necessitating multiple MRI examinations, such as brain tumors, spinal cord abnormalities, or multiple sclerosis.

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