Cranial irradiation impairs intrinsic excitability and synaptic plasticity of hippocampal CA1 pyramidal neurons with implications for cognitive function

Abstract
Radiation therapy is a standard treatment for head and neck tumors. However, patients often exhibit cognitive impairments following radiation therapy. Previous studies have revealed that hippocampal dysfunction, specifically abnormal hippocampal neurogenesis or neuroinflammation, plays a key role in radiation-induced cognitive impairment. However, the long-term effects of radiation with respect to the electrophysiological adaptation of hippocampal neurons remain poorly characterized. We found that mice exhibited cognitive impairment 3 months after undergoing 10 minutes of cranial irradiation at a dose rate of 3 Gy/min. Furthermore, we observed a remarkable reduction in spike firing and excitatory synaptic input, as well as greatly enhanced inhibitory inputs, in hippocampal CA1 pyramidal neurons. Corresponding to the electrophysiological adaptation, we found reduced expression of synaptic plasticity marker VGLUT1 and increased expression of VGAT. Furthermore, in irradiated mice, long-term potentiation in the hippocampus was weakened and GLU1 expression was inhibited. These findings suggest that radiation can impair intrinsic excitability and synaptic plasticity in hippocampal CA1 pyramidal neurons.

Key Words: GABA-mediated hyperfunction; GluR; intrinsic excitability; long-term potentiation; radiation-induced cognitive impairment; spontaneous excitatory postsynaptic currents; spontaneous inhibitory postsynaptic currents; synaptic plasticity; type I vesicular glutamate transporter; vesicular GABA transporter; whole-cell patch clamp recording

Introduction
Radiation therapy is included in established therapeutic protocols used to treat multiple types of head and neck tumors (McTyre et al., 2013; Woonikoko et al., 2014). While cranial radiotherapy has been proven to significantly extend the survival rate of cancer patients, the treatment is routinely associated with serious complications, including cognitive impairment. Indeed, 6 months to 1 year after radiation, 50–90% of patients exhibit cognitive dysfunction that severely affects their quality of life (Greene-Schloesser et al., 2013; Makale et al., 2017). However, the mechanisms by which radiation induces cognitive dysfunction have not been thoroughly elucidated.

The hippocampus has long been considered a pivotal brain area for learning and memory (Bartsch and Wulff, 2015; Wang et al., 2020, 2021; Xue et al., 2021). Structural and functional changes in the hippocampus can result in increased vulnerability to pathological states associated with cognitive deficits (Galvin et al., 1999; von Oertzen et al., 2002; Blum et al., 2012). Notably, patients receiving brain irradiation exhibited deficits in learning and spatial processing, which are related to hippocampal function (Gondi et al., 2010), while hippocampus-avoidance radiotherapy has been found to preserve cognitive function (Andreas and Kundapur, 2015; Brown et al., 2020). Several studies have reported that the hippocampus is vulnerable to radiation, and have linked radiation-induced structural changes in the hippocampus to cognitive decline (Galvin et al., 1999; Rao et al., 2011; Son et al., 2015). Researchers have also reported that deficits in hippocampal neurogenesis (Monje et al., 2002; Zou et al., 2012) and neuroinflammation (Peng et al., 2014; Montay-Gruel et al., 2019) played critical roles in radiation-induced cognitive impairment (Son et al., 2015). However, radiation-induced electrophysiological adaptation in hippocampal neurons has not been well characterized.

Here, we investigated the long-term impact of a single radiation dose of 30 Gy on the intrinsic electrophysiology and synaptic plasticity of hippocampal CA1 pyramidal neurons. Our findings provide new insights regarding the pathogenic mechanisms underlying radiation-induced cognitive deficits.

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Materials and Methods

Animals

Adult male mice were separated randomly into control and radiation groups. Cranial irradiation of the mice was performed using a 6 MV 8-axis linear accelerator (Elekta, Stockholm, Sweden). Anesthetized mice were fixed on a custom-designed platform. The mice were positioned such that the treatment field extended from the post-cranial line to the post-aurem line (Xu et al., 2015). Mice were randomly assigned to experimental groups and the control group was not allowed to die within 60 seconds of irradiation. A total of 22 mice received irradiation and 18 mice underwent a sham operation, and the mice came from three separate cohorts. In the second week, the mice were placed in the group treated with citrate buffer and 100 mg/kg pentobarbital sodium (100 mg/kg/pentobarbital sodium). The mice were allowed to explore the chamber for a total time of 10 minutes after the 60-second lethal injection. The amount of time spent engaged in exploration on days 2 and 3 was measured by a highly experienced observer (JX) who was blinded to the experimental groups. The experimental chamber and objects were cleaned with 70% ethanol to reduce the influence of mouse odor between the trials. The mice were allowed to explore the chamber for a total time of 10 minutes (day 1). On day 2, the animals were exposed to two identical objects in the chamber and given a total exploration time of 10 minutes. On day 3, one of the two objects was relocated to a new position and the animals were allowed to explore the chamber for a total time of 10 minutes. The mice did not explore the two objects for at least 20 seconds within the 10-minute period on day 2 or 3 were removed from the analysis. Throughout the entire experiment, an overhead camera was used to record the behavior of the mice in the chamber. The time spent engaged in exploration on days 2 and 3 was measured by a highly experienced observer (JX) who was blinded to the experimental groups. The experimental chamber and objects were cleaned with 70% ethanol to reduce the influence of mouse odor between the trials. The mice were allowed to explore the chamber for a total time of 10 minutes during which the mice sniffed each object (2 cm within the object with the nose angled directly toward the object). Recognition ratios were calculated as the amount of time spent sniffing an object, divided by the total time spent exploring both objects.

Morris water maze

To examine the spatial memory of mice, we performed the Morris water maze (MWM) as previously reported (Vorhees and Williams, 2006). A circular pool (110 cm in diameter) was filled with water that was made opaque using non-toxic white paint. The temperature of the pool was set at 20–22°C. In the training phase, which took 5 days, the mice were trained to find a hidden platform (23 cm x 33 cm x 200 cm) containing the hippocampus were selected and washed with PBS three times. Sections from mice after anesthetization via 1% pentobarbital (100 mg/kg, intraperitoneal injection). Brains were dissected from mice after anesthetization via 1% pentobarbital (100 mg/kg, intraperitoneal injection). Brains were dissected into ice-cold oxygenated modified artificial cerebrospinal fluid (in mM: 250 sucrose, 10 NaHCO3, 10 MgSO4. 7 H2O, 1.3 NaH2PO4, and 0.2 CaCl2, incubated with 95% O2 and 5% CO2). Coronal hippocampal slices (400 μm thick) were cut using a vibrating microtome (Leica, Wetzlar, Germany, VT-1200S) and incubated at 34°C for half an hour in oxygenated regular artificial cerebrospinal fluid (in mM: 126 NaCl, 26 NaHCO3, 10 glucose, 3 KCl, 2 CaCl2, 1.25 NaH2PO4, and 1 MgSO4). After incubation, the slices were washed three times with artificial cerebrospinal fluid (ACSF) at 32°C–34°C. Electrophysiological data were recorded using a MultiClamp700B amplifier and analyzed with PClamp software (Molecular Devices, San Jose, CA, USA) with a 250 ms time constant and digitization at 10 kHz using Digit4 [Molecular Devices].

For LTP recording, field excitatory postsynaptic potentials (fEPSPs) from the CA1 stratum radiatum were recorded following stimulation of Schaffer collaterals using a two-concentric bipolar stimulating electrode (FHC, Bowdoin, ME, USA). We defined the strength of synaptic transmission as the initial (10–60% rising phase) slope of the fEPSPs. LTP was induced by a 100-Hz stimulation with 50 pulses, and signals were recorded for 60 minutes. The level of LTP was determined by the average fEPSP slope during the last 30 seconds of recording after tetanic stimulation.

To measure the intrinsic excitability of CA1 pyramidal neurons, cells were viewed and selected using an upright microscope (ECLIPSE FN1, Nikon, Tokyo). The pool was filled with a 40% water with a 40% water in a 4% paraformaldehyde solution (in mM: 15 Cs-Meth, 10 KCl, 0.2 EGTA, 2 QX-314, 4 ATP-Mg, 0.3 GTP-Na, and 20 phosphocreatine, pH 7.3), with an mOsm of 290–300. Whole-cell recordings were performed using the whole-clamp technique. The mouse brains were infused with 2μl of depolarizing currents in the presence of 20 μM CNQX (a competitive α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainate receptor antagonist), 100 μM DL-APV (a N-methyl-D-aspartic acid (NMDA) glutamate receptor antagonist), and 20 μM bicuculline. The threshold of an action potential (AP) was calculated as the depolarization at which the neuron fired. The rheobase was determined by performing a series of current injections and recording the current that elicited the first spike. For recording spontaneous excitatory postsynaptic currents (sEPSCs), the neuronal membrane potential was held at –70 mV (using the voltage-clamp technique) in 20 μM bicuculline to block gamma-aminobutyric acid type B (GABA_B) receptors. For recording spontaneous inhibitory postsynaptic currents (sIPSCs), the neuronal membrane potential was held at 0 mV (using the voltage-clamp technique) in 20 μM CNQX and 50 μM DL-APV to block AMPA receptors and NMDA receptors. The pipettes were filled with solution (in mM: 135 Cs-Meth, 10 KCl, 0.2 EGTA, 2 QX-314, 4 ATP-Mg, 0.3 GTP-Na, and 20 phosphocreatine, pH 7.3), with an mOsm of 290–300. We did not analyze sEPSCs or sIPSCs if the mean current was greater than 0.5 mV, or if a 0.5 mV increase in the series resistance of the neurons was greater than 20%. Datasets were attained using pClAMP10.7 (Molecular Devices), and assessed via Clampfit 10.7 software (Molecular Devices) and Mini Analysis software (Synaptosoft Inc., Leonia, NJ, USA).

Immunofluorescence staining

To examine molecular synaptic plasticity in CA1, the brains were collected from mice after anesthesia via 1% pentobarbital (100 mg/kg, intraperitoneal injection). Cardiac arrest was induced by a 4% paraformaldehyde. The brains were post-fixed in 4% paraformaldehyde overnight and then transferred to 20% sucrose in phosphate buffered saline (PBS), followed by 30% sucrose in PBS until they sank to the bottom of the container. We prepared 30-μm-thick coronal sections using a microtome (NX50, Thermo Waltham, MA, USA). Brain slices containing the hippocampus were selected and washed with PBS three times for 5 minutes. After that, sections were incubated with 5% normal donkey serum (Beyotime Biotechnology, Shanghai, China) with 0.4% Triton X-100 for 1 hour at room temperature. After blocking, slices were incubated with a rabbit polyclonal antibody against vesicular GABA transporter (VGAT; 1:500, Cat# 135304, RRID: AB_887878, SynSy) in 1% normal donkey serum and then transferred to the electrophysiological recording area. Brains were dissected using a vibratome (Leica, Heerbrugg, Switzerland) and then fixed in 4% paraformaldehyde solution (in mM: 0.2 CaCl2, incubated with 95% O2 and 5% CO2). Coronal hippocampal slices (400 μm thick) were cut using a vibrating microtome (Leica, Wetzlar, Germany, VT-1200S) and incubated at 34°C for half an hour in oxygenated regular artificial cerebrospinal fluid (in mM: 126 NaCl, 26 NaHCO3, 10 glucose, 3 KCl, 2 CaCl2, 1.25 NaH2PO4, and 1 MgSO4). After incubation, the slices were washed three times with artificial cerebrospinal fluid (ACSF) at 32°C–34°C. Electrophysiological data were recorded using a MultiClamp700B amplifier and analyzed with PClamp software (Molecular Devices, San Jose, CA, USA) with a 250 ms time constant and digitization at 10 kHz using Digital 4 [Molecular Devices].

Western blot assay

The electrophysiological recordings revealed a significant change in synaptic plasticity. Accordingly, we examined the changes in the expression of the associated markers, including neurotransmitter vesicular transporters (VGAT and VGLUT1) and neurotransmitter receptors including AMPAR (Glur1 and
Radiation reduces spontaneous excitatory transmission but increases spontaneous inhibitory transmission to hippocampal CA1 pyramidal neurons

To examine radiation-associated alterations in the excitatory input to hippocampal CA1 pyramidal neurons, we recorded AMPA receptor (AMPA)-mediated sPSCs ($V_{m} = −70$ mV) (Figure 3A). The results showed that compared with the control group, the mean sPSC frequency but not the amplitude in the radiation group was considerably reduced (frequency: $P = 0.018$, amplitude: $P = 0.7038$, Figure 3B). We also recorded GABA receptor-mediated sIPSCs ($V_{m} = 0$ mV) (Figure 3C) to enable a functional analysis of inhibitory synapses. We observed a significant enhancement in the mean frequency but not the amplitude of sIPSCs in the radiation group compared with the control group ($P = 0.0302$, amplitude: $P = 0.7725$, Figure 3D). All of these findings suggest that radiation reduced excitatory synaptic input and enhanced inhibitory input to hippocampal CA1 pyramidal neurons.

Radiation impairs hippocampal LTP

LTP is characterized by the persistent strengthening of synaptic activities, and is thought to be associated with learning and memory (Tittley et al., 2017). To determine the impact of radiation on synaptic plasticity, we recorded EPSPs at Schaffer collaterals to CA1 synapses (Figure 4A). The input-output current relationship of the radiation group was significantly lower than that of the control group ($P = 0.0045$) (Figure 4B). We then recorded LTP for 60 minutes following one train of high-frequency stimulation (100 Hz with 50 pulses) (Figure 4C). In the radiation group, we observed a significant deficit in the LTP slope in the last 10 minutes compared with the control mice ($P = 0.0284$, Figure 4D). Our results indicate that exposure to cranial irradiation impairs LTP/synaptic plasticity in hippocampal CA1 neurons in mice.

Radiation induces molecular alterations in glutamatergic and GABAergic neurons in the hippocampus

Based on the above electrophysiological findings, we investigated the molecular mechanisms responsible for the reduced excitatory and increased inhibitory synaptic input after radiation (Figure 5A). We first investigated the expression levels of VGLUT1 and VGAT in hippocampal CA1 neurons, which are regarded as excitatory and inhibitory presynaptic markers, respectively (Santoro et al., 2017). Immunoblotting revealed a dramatic decline in VGLUT1-positive expression and an increase in VGAT-positive expression in hippocampal CA1 neurons in both the pyramidal layer and stratum radiatum in the radiation versus control group (Figure 5B and C). We then explored whether similar molecular changes were observed in VGLUT1 ($P = 0.0045$) and VGAT ($P = 0.0054$) in the hippocampus via western blot analysis (Figure 5D–G). Western blot analysis of other neurotransmitter receptors that are vital to synaptic plasticity indicated a notable decrease in GluR1 expression ($P = 0.0026$, Figure 5G and Figure 5H) and an increase in GluR2 ($P = 0.7871$; Figure 5G and I). NMDA2a ($P = 0.5459$; Figure 5G and J) or GABA receptor (α1–α6) ($P = 0.9738$; Figure 5G and K) expression in the radiation group compared with the control group. In summary, our data indicate that the radiation mice exhibited significant alterations in synaptic markers of glutamatergic and GABAergic neurons in the hippocampus, providing mechanistic clues regarding the long-term effects of radiation with respect to neuronal dysfunction and cognitive impairment.

Discussion

Although cognitive deterioration after cranial irradiation is extensive and devastating, the mechanisms underlying the cognitive sequelae remain largely undetermined (Greene-Schloesser et al., 2013; Makale et al., 2017). Here, we provide mechanistic changes that occur both within the hippocampus, electrophysiological activity in hippocampal neurons, and associated molecular changes induced by a single 30 Gy dose of radiation in male mice. Our findings offer mechanistic insight regarding cognitive dysfunction following cranial irradiation, suggesting new avenues for therapeutic intervention.

The pathological origins of radiation-induced cognitive impairment depend on multiple factors, including the type of radiation used, radiation dose, and whether the treatment was single or fractionated (Bender, 2012; Boria and Perez-Torres, 2019; de Kruif, 2020), as well as biological sources of variance such as genetic susceptibility (Wang et al., 2019) and sex-based differences (Hinkley et al., 2019; Boria and Perez-Torres, 2020). All of the findings in this study are based on male mice, which represents a limitation regarding the applicability of our data on the effects of cranial irradiation on the intrinsic excitability and synaptic plasticity of female mice. Although most previous animal studies of radiation-induced brain injury have used male animals, recent reports have demonstrated sex differences in the effects of cranial irradiation on cognitive dysfunction, brain necrosis, and spine loss (Hinkley et al., 2019; Boria and Perez-Torres, 2020). Thus, future studies are needed to investigate the influence of sex on the effects of radiation-induced brain injury.
Radiation decreased the sEPSC and increased the sIPSC in hippocampal CA1 pyramidal neurons. Control n.s. | Control Radiation Radiation Radiation

Figure 1 | Mice showed impaired spatial memory performance in the novel object location test (NOL) and the Morris water maze test (MWM) 3 months after a 30 Gy dose of radiation.

(A) Timeline of the NOL and MWM tests. (B) Schematic representation of the NOL. (C) The control and radiation mice were not significantly different in terms of the recognition ratio between objects on day 2 (control: n = 7, independent sample t-test, P = 0.1222; radiation: n = 6, independent sample t-test, P = 0.0772). (D) The control mice had a higher recognition ratio for the relocated object on day 3, while this was not the case for the radiation mice (control: n = 7, independent sample t-test, P < 0.0001; radiation: n = 6, independent sample t-test, P = 0.3335). (E) The escape latency curve during the 5 training days (control/radiation: n = 10/7; two-way repeated measures analysis of variance: time: P < 0.0001; group: P = 0.0009; Sidak’s multiple comparisons test: escape latency on day 2: P < 0.01, day 3: P < 0.01; other days: n.s.). (F) Illustration of the swimming pattern in the control and radiation mice during the probe test. (G) The time spent in each quadrant during the probe test (control/radiation: n = 10/7, independent sample t-test, P = 0.0281). (H) Number of crossings of the location of the hidden platform during the probe test (control/radiation: n = 10/7, independent sample t-test, P = 0.0051). (I, J) The escape latency to the platform (I: control/radiation: n = 10/7; independent sample t-test, P = 0.3369) and the swimming velocity during the cued test (J: control/radiation: n = 10/7, independent sample t-test, P = 0.8728). Data are expressed as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. n.s.: No significance.

Figure 2 | Radiation diminished intrinsic excitability in hippocampal CA1 pyramidal neurons.

(A) Representative firing patterns of hippocampal CA1 pyramidal neurons in the control (black) and irradiated (red) mice in response to depolarizing current injections (100 pA). (B) Averaging the number of spikes fired indicated a decrease in excitability after radiation across a range of current injections (control/radiation: n = 15/15, two-way repeated measures analysis of variance: current injection: P < 0.0001; group: P = 0.0004). (C) Increased rheobase current in the radiation mice (control/radiation: n = 14/15, independent sample t-test, P = 0.0005). (D, E) We found no significant difference in the action potential threshold (D: control/radiation: n = 14/15, independent sample t-test, P = 0.3196) or resting membrane potential (E: control/radiation: n = 14/15, independent sample t-test, P = 0.3197) between the groups. (F) Representative firing pattern of hippocampal CA1 pyramidal neurons from the control (black) and irradiated (red) mice in response to a series of depolarizing current ramps (steps from 0 to 160 pA with a 5-second duration). Scale bars: 20 mV (vertical axis), 200 ms (horizontal axis). (G) The total number of APs was decreased in irradiated mice (control/radiation: n = 14/15, independent sample t-test, P = 0.0006). (H) Radiation delayed the time to the first AP peak (control/radiation: n = 14/15, independent sample t-test, P = 0.0003). Data are expressed as mean ± SEM. ***P < 0.001. AP: Action potential; n.s.: no significance.

Figure 3 | Radiation decreased the sEPSC and increased the sIPSC in hippocampal CA1 pyramidal neurons.

(A) Representative patterns of sEPSC traces in hippocampal CA1 pyramidal neurons from the control (black) and irradiated (red) mice. (B) Cumulative distribution plots and group data (insert) showed a significant decrease in the average frequency of sEPSCs (left) but not the average amplitude (right) in the irradiated mice (frequency: control/radiation: n = 10/12, independent sample t-test, P = 0.0118; amplitude: control/radiation: n = 10/12, independent sample t-test, P = 0.7038). (C) Representative patterns of sIPSC traces from hippocampal CA1 pyramidal neurons from the control (black) and irradiated (red) mice. (D) Cumulative distribution plots and group data (insert) showed a significant enhancement in the average frequency of sIPSCs (left) but not the average amplitude (right) in the irradiated mice (frequency: control/radiation: n = 11/15, independent sample t-test, P = 0.0302; amplitude: control/radiation: n = 11/15, independent sample t-test, P = 0.7725). Data are expressed as mean ± SEM. *P < 0.05. sEPSC: Spontaneous excitatory postsynaptic current; sIPSC: spontaneous inhibitory postsynaptic current.
In the present study, we used a single instance of irradiation with a high dose in our animal model, as per previous reports (Xu et al., 2015). In animal studies and clinical practice, both single instances of irradiation with a high dose and fractionated irradiation with low dose are used (Yang et al., 2017; Milano et al., 2021). We previously reviewed and summarized the pathophysiological responses to radiation-induced brain injury in different animal models (Yang et al., 2017). Generally, fractionated irradiation carries a reduced risk of developing brain injury in comparison to a single high dose of irradiation. For example, a high cumulative dose (40 Gy) delivered via a fractionated irradiation model did not lead to vascular injury or demyelination at 6 weeks post irradiation (Semmler et al., 2013), and no cognitive deficits persisted after 7 months post irradiation (Lee et al., 2012). However, cranial irradiation delivered in a single high dose has several advantages for studying radiation-induced brain injury, including reproducible and stable phenotypes such as long-term cognitive impairment, vascular damage, white matter changes, and giall activation, which occur within weeks post irradiation (Hodges et al., 1998; Liu et al., 2010).

The type of radiation used is also vital to the pathogenesis of radiation-induced cognitive impairment. Linear energy transfer (LET), expressed as keV/μm, is used to describe the amount of energy deposited per unit of length when radiation passes through a material. High-LET radiation induces more damage per absorbed dose than low-LET radiation. Both high-LET radiation and low-LET radiation can uniquely affect neuroinflammation, neurogenesis, and neuronal morphology in animal models (Manda et al., 2009; Cacao and Cucinotta, 2019; Roobol et al., 2020). For example, altered neurogenesis at the early stage post-irradiation in animal models has been found to vary according to the type or dose of irradiation received (Manda et al., 2009; Rivera et al., 2013; Zanni et al., 2018).

To determine how the hippocampus contributes to radiation-induced cognitive deficits, we examined LTP in the CA1 hippocampal region. In contrast to a previous report on LTP in the dentate gyrus (Wu et al., 2012), we found no detectable change in the Schaffer collateral pathway of the hippocampus during the induction phase of LTP. However, we did find improved expression of LTP. Importantly, we found that radiation-induced a decrease in GluR1 expression in the hippocampus. The AMPAR plays a key role in synaptic plasticity (Malinow and Malenka, 2002; Shepherd and Huganir, 2007), and LTP of synaptic strength is reflected through the synaptic insertion of AMPARs, resulting in synaptic strength enhancement and an increase in spine size (Kopec et al., 2007). Since most of the recruited AMPARs have extrasynaptic origins during LTP formation (Malinow and Malenka, 2002; Shepherd and Huganir, 2007), it is critical to understand these changes.
Glutamate released from both presynaptic terminals and GABAergic interneurons (Teichberg et al., 2012) is important for memory consolidation (Bonnin et al., 2013). The mechanisms underlying these processes are not fully understood, but recent studies indicate that GABAergic inhibition may play a crucial role in the regulation of excitability and plasticity in the hippocampus (Nair et al., 2015). In this study, we investigated the effect of a single high-dose irradiation on the intrinsic properties of CA1 pyramidal neurons in an in vitro slice preparation, focusing on the role of GABAergic neurotransmission.

Materials and methods

Animals

All experiments were performed on male Sprague-Dawley rats (250-300 g) housed in a controlled environment with a 12/12-hour light/dark cycle and free access to food and water. The animals were anesthetized with urethane (1.5 g/kg, i.p.) and decapitated. The brains were rapidly removed and placed in an ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 124, KCl 5, MgCl2 1.2, CaCl2 1.2, HEPES 25, and glucose 10. The hippocampi were dissected out and cut into 300-400 μm-thick coronal sections with a vibrating blade microtome. These sections were then transferred to a perfusion chamber (2 ml, 37°C, 95% O2/5% CO2) and allowed to equilibrate for at least 30 min before recording.

Electrophysiological recordings

Whole-cell patch-clamp recordings were obtained using a voltage-clamp amplification system (Axopatch 200B, Molecular Devices, Sunnyvale, CA). The cells were typically held at -70 mV and depolarized with 500 ms-long current steps of varying amplitudes to elicit action potentials. The recordings were filtered at 2 kHz and digitized at 5 kHz for offline analysis using pClamp software (Axon Instruments, Foster City, CA).

GABAergic synaptic responses

GABAergic synaptic responses were evoked by depolarizing current steps that elicited action potentials and were recorded using a fast-scan voltage-clamp technique (Rae et al., 2007). The GABAergic synaptic currents were measured as the difference between the peak inward current during the depolarizing step and the steady-state inward current after the action potential had decayed. The synaptic currents were corrected for the leakage current by subtracting the leak current recorded in parallel with the GABAergic responses.

Results

The effect of irradiation on the intrinsic properties of CA1 pyramidal neurons was investigated using whole-cell patch-clamp recordings. The results showed that a single high-dose irradiation significantly decreased the membrane excitability and calcium currents in CA1 pyramidal neurons, indicating that GABAergic inhibition plays a crucial role in the regulation of excitability and plasticity in the hippocampus. These findings suggest that GABAergic inhibition may be a potential target for the treatment of cognitive impairment associated with high-dose irradiation.

Conclusions

In conclusion, the results of this study provide evidence for the involvement of GABAergic inhibition in the regulation of excitability and plasticity in the hippocampus. These findings suggest that GABAergic inhibition may be a potential target for the treatment of cognitive impairment associated with high-dose irradiation.

Author contributions:

Study design: MYW, WJZ, YT, WI; experiment implementation: MYW, WJZ, YY, SL; data analysis: MYW, WJZ, JX, SQC; manuscript draft: MYW, WJZ, YT, WI, PK, QL. All authors read and approved the final manuscript.

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