### Title

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# Cisplatin induces BDNF downregulation in middle-aged female rat model while BDNF enhancement attenuates cisplatin neurotoxicity

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## Abstract

Cancer-related cognitive impairments (CRCI) are neurological complications associated with cancer treatment, and greatly affect cancer survivors' quality of life. Brain-derived neurotrophic factor (BDNF) plays an essential role in neurogenesis, learning and memory. The reduction of BDNF is associated with the decrease in cognitive function in various neurological disorders. Few pre-clinical studies have reported on the effects of chemotherapy and medical stress on BDNF levels and cognition. The present study aimed to compare the effects of medical stress and cisplatin on serum BDNF levels and cognitive function in 9-month-old female Sprague Dawley rats to age-matched controls. Serum BDNF levels were collected longitudinally during cisplatin treatment, and cognitive function was assessed by novel object recognition (NOR) 14 weeks post-cisplatin initiation. Terminal BDNF levels were collected 24 weeks after cisplatin initiation. In cultured hippocampal neurons, we screened three neuroprotective agents, riluzole (an approved treatment for amyotrophic lateral sclerosis), as well as the ampakines CX546 and CX1739. We assessed dendritic arborization by Sholl analysis and dendritic spine density by quantifying

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CRediT authorship contribution statement

**Naomi Lomeli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Diana C. Pearre:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Maureen Cruz:** Writing – review & editing, Validation, Formal analysis. **Kaijun Di:** Methodology, Data curation. **Joni L. Ricks-Oddie:** Writing – review & editing, Methodology, Formal analysis. **Daniela A. Bota:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors do not have any potential conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2024.114717.

postsynaptic density-95 (PSD-95) puncta. Cisplatin and exposure to medical stress reduced serum BDNF levels and impaired object discrimination in NOR compared to age-matched controls. Pharmacological BDNF augmentation protected neurons against cisplatin-induced reductions in dendritic branching and PSD-95. Ampakines (CX546 and CX1739) and riluzole did not affect the antitumor efficacy of cisplatin *in vitro*. In conclusion, we established the first middle-aged rat model of cisplatin-induced CRCI, assessing the contribution of medical stress and longitudinal changes in BDNF levels on cognitive function, although future studies are warranted to assess the efficacy of BDNF enhancement *in vivo* on synaptic plasticity. Collectively, our results indicate that cancer treatment exerts long-lasting changes in BDNF levels, and support BDNF enhancement as a potential preventative approach to target CRCI with therapeutics that are FDA approved and/or in clinical study for other indications.

**Keywords**

Ampakine; BDNF; Cisplatin; cancer-related cognitive impairments (CRCI Chemobrain); Hippocampal neurons; Neurotoxicity; Ovarian cancer; PSD-95; Riluzole

## 1. Introduction

Cancer survivors often experience neuropsychological changes, including cognitive impairment, anxiety, and depression during and after treatment completion. Cancer-related cognitive impairment (CRCI, commonly referred to as “chemobrain” or “chemo fog”) affects a considerable portion of non-central nervous system (non-CNS) adult cancer survivors. Over 60% of patients report experiencing CRCI during treatment, and up to 35% of cancer survivors experience persistent neurological sequelae years after completing chemotherapy (Janelsins st al., 2014). These impairments reduce survivors’ health-related quality of life (HRQoL), disrupt activities of daily living, and may reduce adherence to treatment plans (Horowitz et al., 2018). Although many advances have been made in the past 20 years within the fields of cancer neuroscience and cancer survivorship to understand the etiology and biological mechanisms underlying cancer and chemotherapy-related neurological complications, no FDA-approved treatments are available for CRCI (Horowitz et al., 2018; Winkler et al., 2023; Lomeli et al., 2021).

Critical gaps of knowledge in the field of CRCI stem from inequalities based on sex and age, as most pre-clinical studies have been conducted with younger male rodents, even though aging increases the risk of cancer (White et al., 2014). The incidence rates of many cancer types (including breast, ovarian, colon, prostate, and lung) rise during midlife. The majority of cancer patients are diagnosed at ages >60 years (Ahles and Root, 2018). CRCI and poor health-related quality of life (HRQoL) are especially prevalent among women treated for ovarian (Correa and Hess, 2012; Hess et al., 2015) and breast cancer (Whittaker et al., 2022; Wefel et al., 2004). Additionally, cancer-related stress, defined as stress related to cancer diagnosis and treatment, is common and can substantially affect long-term HRQoL. However, the impact of cancer-related stress and the contributions to CRCI in aged pre-clinical models remains understudied (Martins-Klein et al., 2021; Lutgendorf et al., 2013).

Brain-derived neurotrophic factor (BDNF) is a crucial mediator of functional and structural plasticity in the central nervous system (CNS); it plays a vital role in the growth and development of neuronal synapses and hippocampal adult neurogenesis. Reductions in neurogenesis and spine density can alter learning and memory and contribute to neurodegeneration (Colucci-D'Amato et al., 2020). Numerous studies have linked the downregulation of BDNF to the pathogenesis of various neurological disorders, including Alzheimer's disease, mild cognitive impairment (MCI), and depression (Phillips et al., 1991; Arosio et al., 2021; Miranda et al., 2019). In addition, elevated serum BDNF levels have been associated with better cognitive outcomes in healthy older adults. In contrast, lower BDNF levels may underlie age-related synaptic loss and cortical and hippocampal atrophy (Gunstad et al., 2008; Erickson et al., 2010). CRCI clinical studies have linked reductions in blood BDNF levels to elevated risks of cognitive impairment in cancer survivors (Ng et al., 2022; Yap et al., 2020; Jehn et al., 2015; Zimmer et al., 2015). Low BDNF levels have been associated with CRCI in female breast cancer survivors (Yap et al., 2020; Yap et al., 2021). Breast and ovarian cancer are commonly diagnosed in peri-menopausal to menopausal women aged 50–69 years. The median age at diagnosis for female breast and ovarian cancer is 62–63 years (Giaquinto et al., 2022) (Torre et al., 2018). Older patients are at a higher risk of experiencing chemotherapy toxicity than younger patients. Chemotherapy dose reductions, delays, and treatment plan modifications are common in the older cancer patient population to minimize toxicity risks (Hamid et al.,2022). Our current understanding of the mechanisms underlying CRCI using chemotherapy regimens for breast and gynecological malignancies has been based on pre-clinical studies conducted in young adult female rodents (aged 2–4 months). A better understanding of CRCI using sex and age-relevant models is essential for identifying biological pathways and developing clinically relevant therapeutic strategies to improve survivors' quality of life.

Our previous studies showed that cisplatin reduced BDNF expression in primary rat hippocampal neurons (Andres et al., 2014). Cisplatin-induced reductions in dendritic branching, spine density, and neural apoptosis were associated with cognitive impairments in rats (Lomeli et al., 2017; Andres et al., 2014). The reductions in dendritic branching in primary hippocampal neurons were irreversible, even after cisplatin's removal, suggesting that chemotherapy's effects may result in persistent neurological changes after treatment completion (Andres et al., 2014). Enhancement of BDNF has been shown to protect neuronal dendritic integrity and improve cognitive function in models of Alzheimer's disease, Parkinson's disease, Huntington's disease, cerebral ischemic stroke, and chronic stress (Miranda et al., 2019; Blurton-Jones et al., 2009; Li et al., 2022). Ampakines, positive allosteric modulators of the AMPA family of postsynaptic ionotropic glutamate receptors, have been shown to reduce memory impairments and rescue synaptic plasticity in models of aging, Huntington's disease, and other neurological disorders (Seese et al., 2020; Simmons et al., 2011; Simmons et al., 2009; Lauterborn et al., 2016). Recently, BDNF enhancement *via* riluzole, an FDA-approved treatment for amyotrophic lateral sclerosis (ALS), has been shown to protect against loss of neurogenesis and reverse doxorubicin-induced cognitive dysfunction in adult female mice (Usmani et al., 2023). In this study, we examine the hypothesis that BDNF enhancement may be a pharmacologically plausible strategy to prevent CRCI. The first aim of this study is to describe an aged female rat model of

cisplatin-induced CRCI and examine the influence of medical stress and cisplatin on serum BDNF levels and cognitive function. The second aim is to conduct an *in vitro* screening of the pharmacological agents, riluzole, and ampakines CX546 and CX1739 in primary rat hippocampal neurons to assess their neuroprotective effects against cisplatin-induced morphological damage. Lastly, the third aim is to screen the oncologic safety of these compounds in conjunction with chemotherapy as potential treatments by evaluating their impact on cisplatin's anti-cancer efficacy in human ovarian cancer cell lines.

## 2. Materials and methods

### 2.1. Animals

Animal studies were performed in accordance with the guidelines established by the NIH and the Institutional Animal Care and Use Committee (IACUC) of the University of California, Irvine. All animal experiments were approved by IACUC (AUP 22–097). All the data were generated using Sprague Dawley rats (Charles River Laboratories). Rats were group housed (4 rats per cage) or housed in pairs, kept on a standard light-dark cycle (12 h each) at 23 °C ± 1 °C room temperature and 45% ± 10% humidity, and given a standard rodent chow diet (Envigo Teklad 2920×) by the University Laboratory Animal Resources (ULAR).

A pilot study was first conducted in twelve 9-month-old female Sprague Dawley rats to determine the maximum tolerated CDDP regimen in this model. We had previously published that chronic CDDP (5 mg/kg/weekly, i.p.) for four consecutive weeks resulted in cognitive impairments in young adult male Sprague Dawley rats (Lomeli et al., 2017) and therefore administered a starting dose of 5 mg/kg, i.p. This dose resulted in a 14.3% decline in baseline weight, therefore we dose-reduced to 3.5 mg/kg, i.p. which was associated with adverse toxicities (20% weight loss). Rats whose weight dropped by 20% were humanely euthanized. We dose-delayed to allow body weights to stabilize, and determined that 2.5 mg/kg, i.p. every other week (cumulative dose = 17.5 mg/kg) was well tolerated (weight loss < 20%) and proceeded to use this regimen for this study (Suppl. Fig. S1).

In this study, 28 nine-month-old female retired breeder Sprague Dawley rats weighing 409 ± 45 g at the start of the study served as subjects. Rats were randomized into three groups: Control (CON, $n = 8$), Saline (SAL, $n = 8$), and Cisplatin (CDDP, $n = 12$). Rats were injected intraperitoneally with 2.5 mg/kg CDDP (Fresenius Kabi, USA, LLC.) dissolved in 0.9% NaCl every two weeks for a total of 7 cycles and a cumulative dose of 17.5 mg/kg CDDP. The SAL group received 0.9% NaCl, i.p., of equal volume. Mannitol (APP Pharmaceuticals, 250 mg/kg, i.p.) was administered to all SAL and CDDP animals one hour before CDDP administration to minimize renal toxicity and increase diuresis. Control (CON) rats received no injections and were not subject to the medical stress protocol.

### 2.2. Medical stress protocol

Cancer incidence increases with age, and stress related to cancer diagnosis and treatment is common and negatively impacts the mental health and quality of life of patients (Martins-Klein et al., 2021). To examine the effects of cisplatin chemotherapy and iatrogenic stress on

serum BDNF levels, anxiety, and cognitive function in 9-month-old Sprague Dawley retired breeders, we developed a medical stress protocol to model the effects of environmental stress associated with cancer care. Rats were randomized into three groups, healthy controls (CON), saline (SAL), and cisplatin (CDDP). The rats were acclimated to the vivarium for two weeks. CDDP and SAL rats received 5–10 mL of 0.9% saline, s.q., five times a week during each CDDP cycle to reduce dehydration associated with CDDP. Three days post-CDDP administration, blood sampling from the lateral tail vein were used to assess serum BDNF levels in the CDDP and SAL rats. The rats were restrained using a Tailveiner® restrainer (Braintree Scientific). A maximum of two blood draws were attempted per rat per timepoint. Subjects were weighed three times a week during the study. CON rats served as healthy age-matched controls and were not exposed to the medical stress protocol.

## 2.3. BDNF enzyme-linked immunosorbent assay (ELISA)

**2.3.1. Serum BDNF measurements—**To assess the effect of cisplatin on peripheral BDNF levels, blood was collected from the lateral tail vein of SAL and CDDP rats 72 h after each CDDP cycle. Approximately 50–150 μL blood was collected per rat. In the CDDP and SAL groups, blood collection was attempted twice per rat per timepoint. Serum BDNF quantification in Fig. 1H-L represents time points in which at least three serum samples were successfully collected and measured per group. SAL and CDDP serum BDNF levels were compared to the serum BDNF levels of age-matched controls (CON) collected at the terminal time point. Blood was clotted for 30 min at room temperature (25 °C) in 0.6 mL tubes, then centrifuged at 2000g for 15 min at room temperature. Serum (approximately 20–60 μl) was collected and stored at −80 °C for further analysis. Serum concentrations of BDNF were measured using the Human/rat BDNF ELISA kit (Proteintech, KE00096) according to the manufacturer's instructions at a 1:10 dilution. All samples from each subject were assayed simultaneously on the same plate in triplicate to minimize run-to-run variability.

**2.3.2. Hippocampal tissue BDNF measurements—**Rats were deeply anesthetized with Euthasol®. After confirming sedation, the rats were perfused with ice-cold 0.9% saline *via* transcardiac perfusion. The brains were removed, and the hippocampi were snap-frozen on dry ice and stored at −80 °C until sample preparation. Hippocampi tissue was lysed using ice-cold Pierce RIPA Buffer (Thermo Scientific, 89900) containing 2× Phosphatase Arrest (Gbiosciences, 786–450) and Protease Inhibitor (Sigma, P3840) at a weight/volume ratio of 10:1. Samples were passed through a fine gauge needle to break up the tissue, and were incubated on ice for 30 min. Sample extracts were centrifuged at 10,000g for 20 min at 4 °C, and the pellet was discarded. Protein concentration in the tissue lysates was quantified using a DC Protein Assay (Bio-Rad, 5000111). Lysates were aliquoted and stored at −80 °C until further steps. We used the Human/rat BDNF ELISA kit (Proteintech, KE00096). An initial pilot study was performed with serial dilution of tissue lysates to determine the optimal lysate concentration. Once determined, all samples were tested at a 1:100 dilution and run in triplicate on the sample plate. The assay was performed according to the manufacturer's instructions. The BDNF concentration in each sample was normalized to total protein concentration to give the BDNF concentration per milligram of total soluble protein [pg/mg].

## 2.4. Cognitive testing

**2.4.1. Open field test—**Open field testing was conducted in CON, SAL, and CDDP rats, 14 weeks after the initiation of cisplatin chemotherapy (two weeks after cisplatin completion). To determine the effect of cisplatin on anxiogenic behavior after chronic chemotherapy, rats were placed in an open opaque square Plexiglas box arena (60 cm × 60 cm) with 60 cm high walls at the center of the arena and allowed to freely explore for a 10 min session. Rats were removed from the arena at the end of the session, and the arena was cleaned with 70% ethanol and dried before the subsequent session. Each session was recorded by an overhead video camera (Swann Security System) and analyzed manually by a blinded scorer. The arena was divided into a 30 cm × 30 cm center zone and a peripheral zone. The frequency of entrances and time spent in the center zone were quantified, with less time spent in the center zone considered anxiogenic behavior.

**2.4.2. Novel object recognition—**To determine the effect of cisplatin on cognitive function after chronic chemotherapy, all rats were examined on the novel object recognition (NOR) task 14 weeks after the initiation of cisplatin chemotherapy. We conducted NOR testing as previously described (Lomeli et al., 2017). Rats were placed in an open opaque Plexiglas box arena (60 cm × 60 cm) with 60 cm high walls containing two identical objects. Each rat explored the arena for 5 min per day for two consecutive days. The 5-min test trial was given 24 h later, during which the rat was presented with one of the familiar objects from the training phase paired with a new object. Total exploration time, time spent exploring each object (seconds), and the discrimination ratio (time spent exploring the novel object/total exploration time) were quantified.

## 2.5. Primary rat hippocampal neuron cultures

As previously described, hippocampal neuron cultures are prepared from postnatal day 0 (P0) Sprague Dawley pups (Lomeli et al., 2017; Andres et al., 2014; Lomeli et al., 2020). Cells were plated at a $7.5 \times 10^3$ cells/coverslip density on 12 mm coverslips (Chemglass Life Sciences) pre-coated with 0.2 mg/mL poly-D-lysine (Sigma Aldrich). Cells were maintained in Neurobasal Plus Medium (NBM+) with B-27 Plus (Gibco) at 37 ° C and 5% $CO_2$. On day *in vitro* 3 (DIV3), cultures were treated with 5 μM arabinoside-cytosine (Sigma, C1768) to inhibit glial proliferation and refreshed twice a week with conditioned medium. Mature neurons were used for experiments on 17–21 days *in vitro* (17–21 DIV).

## 2.6. Ovarian cancer cell lines

The established OVCAR8 and SKOV3.ip1 cell lines were maintained in RPMI 1640 medium with 300 mg/L L-Glutamine (Corning 10–040-CV) containing 10% FBS (Omega Scientific, Inc) and 1× penicillin/streptomycin (Gibco). These cell lines were kindly gifted by Dr. Olga Razorenova.

## 2.7. XTT assay

The cells were seeded at approximately $10 \times 10^3$ cells/well in a final volume of 200 μl in clear 96-well plates. The plates were incubated at 37 °C at 5% CO2 for 72 h at the

specified final concentrations of BDNF/riluzole/CX546/CX1739 ± Cisplatin. Cell viability was determined using the Biotium XTT Cell Viability Kit (Biotium 30007).

### 2.8. Drug application in vitro

*In vitro*, recombinant human/murine/rat BDNF (Peprotech, 450–02) was reconstituted in 0.1% BSA in di$H_2O$ to make 100 μg/mL BDNF stocks and stored at −20 °C. BDNF was diluted to a final concentration as specified in the respective cell culture medium. Riluzole (Sigma, R116-25MG) was dissolved in DMSO to make a 10 mM working solution. Riluzole was freshly prepared immediately before each *in vitro* experiment to minimize loss of efficacy after freeze-thawing. Ampakine CX546 (Tocris, 2980/10) was made into a 100 mM stock by dissolving in DMSO and stored at −20 °C. Ampakine CX1739 was obtained from Cortex Pharmaceuticals. CX1739 was made into 25 mM stocks in 33% (2-HydroxypiOpyl)-β-cyclodextrin (HPCD) (made 1:1 in 0.9% NaCl and distilled $H_2O$) (Sigma, 332607). All treatment groups were exposed to an equal volume of the respective vehicle for all experiments.

### 2.9. Immunocytochemistry (ICC)

Neurons were fixed with ice-cold 4% paraformaldehyde (PFA, Thermo Scientific AC41678–00) in PBS pH 7.4 for 12 min. To evaluate dendritic branching, neurons were immunostained with an antibody against postsynaptic density-95 (PSD-95). The cells were incubated with mouse anti-PSD-95 1:4000 (Thermo Fisher, MA1–046) in 200 μl per well in blocking buffer (0.3% FBS, 0.1% Triton-X in PBS, pH 7.4) at 4 °C. The following day, coverslips were washed with PBS and incubated in goat anti-mouse Alexa Fluor 594 nm 1:500 (Jackson Immuno Research, NC0540445) at room temperature for 1.5 h. Coverslips were mounted on slides using DAPI Fluoromount G (Southern Biotech) mounting medium.

### 2.10. PSD-95 puncta quantification

Dendritic spine density was quantified as the number of PSD-95 positive puncta on dendritic branches. Images were generated by confocal microscopy, Olympus FV3000. 3 μm z-series (0.5 μm steps) images were captured from dendrites that were clearly distinct from dendrites of other neurons and dendritic crossings at 60 × 1.5 zoom (NA 1.42) using an oil-immersion objective. Each experiment included 3 coverslips per treatment group, with 4 dendrites from 2 separate neurons analyzed per coverslip, for a total of 6 neurons, 12 dendrites per treatment group. Spine density was expressed as the number of PSD-95 puncta per 20 μm of dendritic length, extending to 100 μm from the soma. Images were processed for analyses by conversion into 8-bit black and white TIFF files, then scaled for distance per pixel length using Fiji. The distance from the soma was then measured and divided into 20 μm segments. Each individual puncta were considered a separate spine, and the number of PSD-95 puncta localized in clusters was adjusted based on the cluster size.

### 2.11. Sholl analysis

Dendritic branching was evaluated using Sholl analysis. The total dendritic length was measured, and the number of intersections between branches and concentric circles at increasing 20 μm segments from the soma was quantified. Images were generated by

confocal microscopy, Olympus FV3000. 8 μm z-series (1 μm steps) images were captured at 20× (NA 0.75), spanning entire neurons. Each experiment included 3 coverslips per treatment group, with 3 neurons imaged per coverslip, for a total of 9 neurons per treatment group. Images were processed for analyses by conversion into 8-bit black and white TIFF files, and concentric circles were overlayed on the images using the Concentric Circles plug-in in Fiji.

### 2.12. BDNF qPCR assay

Total RNA was extracted using RNeasy Mini Kit (Qiagen, Germantown, MD, USA), and cDNA was generated using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) as previously described (Andres et al., 2014). Quantitative PCR reactions (iQTM SYBR Green Supermix, Bio-Rad) were done using a Bio-Rad CFX96 Real-time System. *BDNF* gene expression levels were normalized to *GPADH* expression levels. The sequences for rat BDNF and GAPDH primer sets were: *BDNFCDS* forward 5′-AAACGTCCACGGACAAGGCA-3′, reverse 5′-TTCTGGTCCTCATCCAGCAGC-3′; *GAPDH* forward 5′-CCTTCATTGACCTCAACTACAT-3′, reverse 5′-CCAAAGTTGTCATGGATGACC-3′. The primers were ordered from IDT, Integrated Device Technology, Inc., Coralville, Iowa, USA.

### 2.13. Systematic analysis and statistical considerations

Each experiment included three replicates per treatment group and was repeated twice. All imaging and analysis of PSD-95 and dendritic branching and behavioral testing were performed by two researchers blinded to the experimental conditions. All biochemical, behavioral, and imaging experiments were analyzed using one-way ANOVA or two-way repeated measures (RM)-ANOVA, followed by Tukey's *post hoc* multiple comparisons test. All data are presented as the mean ± SEM, and significance levels were set at 0.05. Data were analyzed using GraphPad Prism 8.0 Software.

## 3. Results

### 3.1. Cisplatin and medical stress decreases serum BDNF and impairs cognitive function in female rats

To examine the effects of CDDP and medical stress-associated changes in serum BDNF levels and cognitive function, we first developed a CDDP regimen and medical stress protocol to induce CRCI in middle-aged female Sprague Dawley rats. Rats were randomly assigned to one of three groups: healthy-aged, matched controls (CON, $n = 8$), saline (SAL, $n = 8$), and cisplatin (CDDP, $n = 12$). SAL and CDDP groups were exposed to the medical stress protocol which models the clinical conditions cancer patients are exposed to during cancer treatment (Crona et al., 2017). Blood samples were collected 72 h following CDDP injections in the SAL and CDDP groups. In addition, SAL and CDDP rats received 5–10 mL of 0.9% saline, s.q., for 5 days as hydration therapy following CDDP injections. The CON group did not receive any injections and were not subjected to the medical stress protocol.

Every other week, for 13 weeks, nine-month-old female rats received an intraperitoneal injection of CDDP (2.5 mg/kg) dissolved in 0.9% NaCl, or an equal volume of 0.9%

NaCl (cumulative CDDP dose of 17.5 mg/kg, i.p., Fig. 1A). This CDDP regimen was well-tolerated with no adverse toxicities (i.e., weight loss >20%, death, Fig. 1B, Suppl. Fig. S1A).

To determine the tolerable clinically relevant CDDP regimen in this model that induced CRCI without adverse toxicities (*i.e.*, weight loss >20%, death), we had first conducted a pilot study using a chronic CDDP regimen of 5 mg/kg, i.p., every other week ($n = 12$). The first dose resulted in a 14.3% decline in baseline weight, and significant toxicity was observed after the second cycle; therefore, we dose-reduced and dose-delayed to allow weights to stabilize, and we determined that 2.5 mg/kg could be administered biweekly with minimal toxicity (Suppl. Fig. S1 B, C). The regimen used in this study (2.5 mg/kg/biweekly) for 13 weeks is lower than that which induced CRCI in 2-month-old male Sprague Dawley rats (5 mg/kg, i.p.) weekly for 4 weeks (Lomeli et al., 2017). CDDP caused an additive reduction in body weight over time ($F_{(16, 198)} = 15.03$, $P < 0.0001$, Fig. 1B). Renal toxicity is a major dose-limiting side effect of CDDP. Compared to younger patients, the incidence of cisplatin-induced nephrotoxicity is higher in older patients, which may influence clinicians' decision to use a low-dose regimen of CDDP or switch to a more tolerable platinum agent, carboplatin (Duan et al., 2020). Modifications to cancer treatment plans, including dose delays and dose reductions are common in older adults (Hamid et al., 2022), which validates our CDDP dosing regimen in our model. Our objective was to assess the effects of medical stress on serum BDNF levels, anxiogenic behavior, and novel object recognition on rats receiving a CDDP regimen or saline compared to healthy controls.

Two weeks after CDDP treatment completion, anxiogenic behavior was assessed using the open field test (OFT) and the novel object recognition (NOR) task to assess cognitive performance. Open field activity was observed on day 1 of the habituation phase. There was no significant difference in the time spent in the center zone of the arena ($F_{(2,25)} = 0.01957$, $P = 0.9806$) or the number of entrances to the center zone ($F_{(2,25)} = 0.6460$, $P = 0.5327$) between the groups (Fig. 1C, D), which suggests the absence of anxiogenic behavior during the cognitive testing. During the NOR test phase, there was no difference in the total time spent exploring the novel and familiar object between the experimental groups (Fig. 1E). Comparison of the time spent exploring the novel object compared to the familiar object revealed significant differences in object exploration in the CON rats ($P = 0.0069$), but not for the SAL ($P = 0.1256$) or CDDP-treated rats ($P = 0.2118$, Fig. 1F). A one-way ANOVA revealed a significant overall treatment effect between groups ($F_{(2,25)} = 5.897$, $P = 0.0080$). The discrimination ratio was calculated as [(time spent exploring the novel object)/(total exploration time)] for each subject. The CDDP rats showed a significantly reduced discrimination ratio compared to the CON rats, who were not exposed to the medical stress protocol ($P = 0.0057$). There was no significant difference in discrimination between CDDP (DR = 0.533) and SAL (DR = 0.589, $P = 0.3309$) groups. SAL rats showed a trend towards reduced discrimination compared to the CON rats ($P = 0.1872$, Fig. 1G). These data suggest that CDDP results in cognitive deficits and that medical stress associated with cancer treatment may influence these impairments.

### 3.2. Effect of chronic cisplatin and medical stress on serum BDNF levels

Brain-Derived Neurotrophic Factor (BDNF), a member of the neurotrophin family, plays a crucial role in promoting neuronal survival and growth, synaptogenesis, and is essential to learning and memory (Miranda et al., 2019). Past studies in rat models have shown that cisplatin treatment reduces BDNF levels in the hippocampus (Andres et al., 2014; Abdelkader et al., 2017). Chronic stress is associated with loss of dendritic arborization and spines, changes in synaptic plasticity, and cognitive impairment (Chen et al., 2008). Although multiple factors are involved in stress-induced changes in the plasticity and integrity of excitatory synapses (Andres et al., 2013), chronic stress induces transient downregulation of BDNF mRNA levels in the rat hippocampus (Murakami et al., 2005). To examine the longitudinal effects of cisplatin and medical stress on serum BDNF levels and their association with the behavioral outcomes observed in the SAL and CDDP-treated rats, we collected serum samples 72 h after each CDDP cycle and 10 weeks post-CDDP completion and measured BDNF levels by ELISA (Fig. 2A-E). We observed trending decreases in serum BDNF levels in SAL and CDDP-treated rats after CDDP Cycle 1. One-way ANOVA revealed a significant overall treatment effect between groups ($F_{(2,18)} = 4.640$, $P = 0.0237$) following CDDP Cycle 4 (Fig. 2C). Serum BDNF levels were significantly lower in CDDP-treated rats (609.2 pg/mL ± 161.1 pg/mL) compared to CON (1412 pg/mL ± 202 pg/mL), 72 h following CDDP Cycle 4 ($P = 0.0257$). Serum BDNF levels were also decreased in SAL-treated rats (726.5 pg/mL ± 304.7 pg/mL) but not significantly compared to CON (Fig. 2C, $P = 0.1065$). Notably, the serum BDNF levels partially recovered in SAL rats (1190 pg/mL ± 290.1 pg/mL), while the levels remained low in CDDP-treated rats (775.2 pg/mL ± 187 pg/mL) compared to CON (1412 pg/mL ± 202 pg/mL) 10 weeks following CDDP completion (Fig. 2E). In parallel, total BDNF protein levels were evaluated in hippocampal tissue collected 10 weeks following CDDP completion. However, we found that BDNF hippocampal levels in the SAL rats (1354 pg/mg ± 130.4 pg/mg) and CDDP rats (1381 pg/mg ± 105 pg/mg), were not significantly different compared to CON (1028 pg/mg ± 90.96 pg/mg) levels at this timepoint (Fig. 2F; $F_{(2,21)} = 2.984$, $P = 0.0723$).

Although not statistically significant, these trends suggest that medical stress induces transient but clinically meaningful reductions in serum BDNF that may resolve following treatment completion. However, the effects of CDDP on serum BDNF levels are dose-dependent and last long following treatment discontinuation. Additionally, in our study we found that BDNF levels in the hippocampus returned to control levels 10 weeks after CDDP discontinuation and may not be reflective of serum BDNF protein levels long-term following treatment cessation.

### 3.3. In vitro BDNF application prevents cisplatin-induced reductions in dendritic branching and PSD-95 puncta density

Reductions in BDNF expression are associated with cognitive impairment in multiple models of CRCI (Usmani et al., 2023; Geraghty et al., 2019; Taha et al., 2023). We previously reported that cisplatin significantly reduces BDNF mRNA levels. This reduction in BDNF expression was accompanied by reductions in postsynaptic density-95 (PSD-95), a surrogate marker of dendritic spines, in rat hippocampal neurons following exposure to 0.1 μM, 1 μM cisplatin at short (2 h) and chronic (24 h, 48 h) time-points (Lomeli

et al., 2017; Andres et al., 2014). To test directly whether BDNF supplementation could prevent cisplatin-induced reductions in dendritic branching and spine density, we assessed the effect of CDDP and BDNF administration on dendritic arborization *via* Sholl analysis and expression of PSD-95 in mature rat cultured hippocampal neurons.

We measured the number of dendritic branch points from the soma in hippocampal neurons exposed to 1 μM cisplatin for 24 h (Fig. 3A, B). Sholl analysis showed that CDDP significantly reduced the number of dendritic intersections compared to vehicle ($P = 0.0034$), which was prevented by co-treatment with BDNF. Two-way repeated measures ANOVA revealed a significant interaction across all treatment groups, $F_{(45,360)} = 2.619$, $P < 0.0001$. The CDDP-induced loss of dendritic arborization was also accompanied by a reduction in PSD-95 density compared to vehicle ($P < 0.0012$), which was prevented by BDNF ($F_{(3,20)} = 12.77$, $P < 0.0001$, Fig. 3C, D). Cisplatin-associated loss of dendritic arborization and PSD-95 puncta density was prevented by exogenous application of BDNF, which suggests that enhancing hippocampal BDNF levels may prevent cisplatin-induced morphological damage.

### 3.4. In vitro, riluzole prevents cisplatin-induced reductions in dendritic arborization and PSD-95 density

Given the critical role of BDNF in regulating synaptic plasticity and the association of low BDNF levels with the development of CRCI, we next sought to examine whether pharmacological enhancement of BDNF may be a novel therapeutic strategy to prevent CRCI. We conducted a screening study of three neuroprotective pharmacological agents that have been shown to improve cognitive function: riluzole (RZ), ampakine CX546, and ampakine CX1739.

Riluzole was approved in 1995 by the Food and Drug Administration (FDA) for the treatment of amyotrophic lateral sclerosis (ALS). Riluzole is an anticonvulsant that blocks voltage-gated $Na^+$ channels and has been shown to enhance BDNF levels in the hippocampus (Katoh-Semba et al., 2002; Bellingham, 2011). We first conducted a dose-response experiment to determine the effects of RZ on dendritic arborization and PSD-95 density. We tested four doses 1 μM, 2.5 μM, 5 μM, and 10 μM RZ (Suppl. Fig. 2A, B). Riluzole alone did not significantly change dendritic arborization ($F_{(4,32)} = 1.720$, $P = 0.1698$) or PSD-95 density ($F_{(4,25)} = 0.6280$, $P = 0.6470$) compared to vehicle at any of the tested doses at all distance points along the dendrites. In the presence of RZ (1 h pre-treatment), CDDP no longer reduced dendritic arborization ($F_{(45,480)} = 2.559$, $P < 0.001$, Fig. 3E, F). RZ also protected dendritic spines from CDDP-induced PSD-95 loss ($F_{(12,80)} = 1.899$, $P = 0.0467$, Fig. 3G, H).

### 3.5. In vitro, ampakines CX546 and CX1739 protect against cisplatin-induced reductions in dendritic arborization and PSD-95 density

Ampakines are a structurally diverse family of small molecules that positively modulate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors and enhance fast, excitatory transmission in the CNS (Lynch, 2006). Ampakines have been shown to up-regulate BDNF, rescue synaptic plasticity, and ameliorate cognitive

impairments in rodent models of neurological disorders (Seese et al., 2020; Simmons et al., 2009; Lauterborn et al., 2016; Kramár et al., 2012). Ampakines increase fast excitatory synaptic currents by modulating the receptor rate constants for transmitter binding, channel opening, and desensitization (Lauterborn et al., 2000). Neuronal BDNF is induced by AMPAR activation and patterns of electrical activity, such as depolarization, high-frequency stimulation, or theta-burst stimulation (Edelmann et al., 2014). Both ampakines, CX546 and CX1739, have been shown to potentiate excitatory signaling, but only CX546 increases BDNF mRNA and protein levels (Lauterborn et al., 2000; Lauterborn et al., 2003; Clarkson et al., 2011). We previously showed that CDDP induced a pronounced reduction in BDNF mRNA levels in primary hippocampal neurons as early as 2 h after treatment (Andres et al., 2014). We next asked whether ampakine CX546 administration could prevent CDDP-induced reductions in BDNF expression (Fig. 4A). 0.1 μM CDDP significantly reduced BDNF mRNA expression levels compared to VEH ($P = 0.0032$) 24 h post-treatment. Ampakine CX546 alone (50 μM) significantly increased BDNF levels by ~6-fold compared to VEH ($P < 0.0001$). CX546 significantly prevented CDDP-induced reductions in BDNF levels compared to CDDP alone ($P < 0.0001$). One-way ANOVA revealed a significant treatment effect between CDDP and CX546, ($F_{(3,8)} = 407.0$, $P < 0.0001$). Co-treatment with ampakine CX546 significantly prevented CDDP-induced reductions in dendritic arborizations ($F_{(45,360)} = 4.570$, $P < 0.0001$, Fig. 4B, C) and PSD-95 puncta density ($F_{(12,80)} = 2.864$, $P = 0.0025$, Fig. 4D, E). Additional studies examining the effect of a different ampakine, CX1739, showed that CX1739 prevented CDDP-induced PSD-95 loss ($F_{(12,80)} = 6.328$, $P < 0.0001$, Fig. 4F, G).

While BDNF has been widely recognized for its role in the development, migration, differentiation, and survival of fetal neurons, the effects of BDNF enhancement on undifferentiated neural stem/progenitor cells (NSC) exposed to cisplatin have not been fully examined. We conducted an *in vitro* screening of graded doses of BDNF (100–300 ng/ mL), riluzole (1–10 μM), and ampakine CX546 (10–100 μM) on rat embryonic hippocampus-derived NSCs (Andres et al., 2014) treated with or without cisplatin exposure (Suppl. Fig. 3). We found that BDNF enhancement did not prevent cisplatin-induced reductions in viability following 72 h of treatment.

### 3.6. In vitro, screening of BDNF-enhancing compounds in human ovarian cancer cell lines

To examine whether BDNF alters cancer cell viability *in vitro*, we assessed the viability of two human ovarian cancer cell lines, OVCAR8 and SKOV3.ip1, exposed to 10 μM CDDP with or without increasing concentrations of BDNF, riluzole, CX546, or CX1739, for 72 h (Fig. 5). CDDP (10 μM) reduced OVCAR8 viability to 45.23% ± 0.9763% and 30.75% ± 0.5271% in SKOV3.ip1 at 72 h. BDNF alone had no effect on OVCAR8 and SKOV3.ip1 viability at BDNF concentrations ranging between 50 ng/mL to 200 ng/mL, with 300 ng/mL BDNF inducing a 4.84% ± 0.2447% reduction in SKOV3.ip1 viability (Fig. 5A). BDNF did not reduce CDDP's anti-cancer efficacy in cells co-treated with BDNF and CDDP at any of the doses tested. Riluzole alone had no effect on cell viability compared to the vehicle control (Fig. 5B). The addition of riluzole (2.5–20 μM) to cisplatin-treated cancer cells did not affect cisplatin's killing efficacy (Fig. 5B). At the highest concentration tested, 300

μM, CX546 alone induced a small but statistically significant increase in OVCAR8 (3.9% ± 0.3885%) and SKOV3.ip1 (7.6% ± 0.5028%) viability compared to the vehicle control ($P < 0.001$, Fig. 5C). Co-treatment with CX546 reduced CDDP's anti-cancer efficacy ($P < 0.001$). The addition of 50 μM CX 546 to CDDP-treated OVCAR8 and SKOV3.ip1 increased cell viability to 10.79% and 10.88% compared to 10 μM CDDP alone, respectively ($P < 0.001$, $P < 0.001$, Fig. 5C). This effect was dose-dependent in the SKOV3.ip1 cell line as co-treatment with 300 μM CX546 dose exerted a 30.48% increase in viability compared to 10 μM CDDP alone ($P < 0.001$, Fig. 5C). Ampakine CX1739 exerted an increase in viability, with 300 μM CX1739 exerting a significant increase in OVCAR8 (24.2% ± 2.715%, $P = 0.0432$) viability compared to the vehicle control (Fig. 5D). Unlike CX546, CX1739 did not alter CDDP's anti-cancer efficacy when co-applied with CDDP to ovarian cancer cells.

## 4. Discussion

The connection between BDNF and cognitive function in CRCI has come into focus in recent years. A recent systematic review assessing the relationship between BDNF plasma/serum levels and neurocognitive outcomes in cancer patients and survivors across multiple cancer types found a positive correlation between BDNF levels and cognitive function across various clinical studies (Ng et al., 2022), with low BDNF levels during chemotherapy treatment linked to long-term cognitive dysfunction. In rodent models, reductions in BDNF levels were observed in the hippocampus following 5-FU (Mustafa et al., 2008), cisplatin (Saral et al., 2023), doxorubicin (Usmani et al., 2023; Park et al., 2018), and methotrexate (Taha et al., 2023) systemic exposure. Our earlier findings that cisplatin induced loss of dendritic spines and arborization and reduced BDNF expression in primary hippocampal neurons led us to test the hypothesis that loss of BDNF contributes to CRCI, and BDNF enhancement may mitigate cisplatin-induced neurotoxicity (Andres et al., 2014). This current study provided pre-clinical evidence that cisplatin reduces serum BDNF levels in a female rat model of CRCI. Our study is the first to describe the effects of cisplatin on cognition and BDNF serum levels in a middle-aged female rat model. Notably, compared to a young adult male rat model of CRCI (Lomeli et al., 2017), this model was more sensitive to cisplatin-induced toxicities. The cisplatin regimen (2.5 mg/kg, i.p.) every other week for seven cycles is comparable to the clinical regimens for ovarian and other gynecological cancers (Ozols et al., 2006; Ozols and Young, 1985).

Cognitive impairments and attention difficulties are grave concerns for cancer survivors. Cisplatin and other platinum agents (oxaliplatin, carboplatin) have been widely used in the past 40 years in the treatment of several malignancies, including ovarian, lung, testicular, head and neck, and triple-negative breast cancer (Silver et al., 2010; Brown et al., 2019). CDDP binds to DNA, forming DNA-Pt adducts, which results in DNA crosslinking, mitochondrial dysfunction, and apoptosis. Neurological complications following platinum-based chemotherapy are common in ovarian cancer survivors (Correa and Hess, 2012; Hess et al., 2015). Hess et al. assessed cognitive function in women with newly diagnosed ovarian cancer who were prescribed a minimum of six cycles of chemotherapy using a computerized, web-based assessment at three time points: before the first course (baseline), course three, and course six. Of the 27 eligible participants, 92% and 86% demonstrated

impairments at courses three and six, compared to baseline, respectively. Impairment was detected in two or more cognitive domains in over 40% of participants. Correa et al. examined neuropsychological function in long-term survivors of ovarian cancer 5 to 10 years from diagnosis and found that 28% of survivors had impairments in attention, executive function, and memory (Correa and Hess, 2012). The changes in cognitive function experienced by cancer patients are associated with structural and functional changes in the brain. Structural and functional magnetic resonance imaging (MRI) in ovarian cancer patients treated with first-line chemotherapy revealed reductions in grey matter volume and significantly decreased activation in frontal cortical and parietal regions involved in executive functions, compared to healthy age-match controls, 1–4 months post-chemotherapy completion (Correa et al., 2017). These studies provide evidence that the symptoms of CRCI have a biological basis.

Middle-aged female rats (9- to 15-month-old) were used in this study to model the effects of CRCI on an age-relevant rat model for gynecological cancers. Most pre-clinical CRCI studies have used young (2- to 3-month-old) male rats to examine the effects of chemotherapy on cognition, which does not consider the effect of age, sex, or chemotherapy regimen that is relevant for this specific cancer patient population (Ossorio-Salazar and D'Hooge, 2023). To our knowledge, no pre-clinical studies have compared the long-term effect of cancer-associated medical stress (clinic visits, procedures, chemotherapy administration) on cognition in CRCI rodent models. A cancer diagnosis, followed by a prolonged period of cancer treatment, are stressful life-altering experiences that cause emotional distress and may also contribute to the cognitive impairments experienced by cancer survivors (Martins-Klein et al., 2021). We found that the rats exposed to the medical stress protocol who received 0.9% saline (SAL) and those exposed to the medical stress protocol who received cisplatin (CDDP) had decreased serum BDNF levels following cycle 4 (Fig. 2C). Both SAL and CDDP groups had impaired discrimination in the NOR task compared to the healthy-aged, matched controls (CON) two weeks following CDDP completion. Ten weeks post-CDDP completion, the serum BDNF levels in the CDDP group remained low; the CDDP BDNF levels increased by 27.24% compared to the levels after cycle 4 (Fig. 2C, E). In contrast, the serum BDNF levels in the SAL group increased by 63.7% compared to the levels after cycle 4 (Fig. 2C, E). We found that hippocampal total BDNF protein levels were not significantly different in the SAL and CDDP groups compared to the controls 10 weeks after CDDP completion. Various studies have observed significant decreases in hippocampal BDNF mRNA and protein levels shortly after CDDP completion (72 h – 1 week) (Saral et al., 2023; Abdollahzadeh et al., 2022; Jangra et al., 2016) in young male rats. However, long-term measures of hippocampal BDNF levels following chemotherapy completion have not been previously reported. In our study, total BDNF protein levels were measured in the serum and the hippocampus. A plausible explanation for the normalized BDNF protein levels in the hippocampus of CDDP and SAL rats may be due to the upregulation of precursor proBDNF, which is proteolytically cleaved to generate mature BDNF. Mature and pro-BDNF exert opposite effects in the CNS and in the periphery. Mature BDNF promotes survival, differentiation, synaptic plasticity, and long-term potentiation, whereas pro-BDNF is associated with apoptosis, reduced dendritic spine density, and long-term depression (De Vincenti et al., 2019). More studies are warranted to

understand possible differences in proBDNF *vs.* in mBDNF in the hippocampus and serum in rats following CDDP chemotherapy.

Our findings suggest that medical stress may contribute to changes in BDNF and cognitive function during chemotherapy treatment and that increasing BDNF levels *in vivo* may provide a therapeutic strategy to prevent CRCI. To our knowledge, this is the first pre-clinical study evaluating the effects of medical stress on cognition. A limitation of our study was that due to the small sample size, challenges in consistently collecting blood samples from the same rat at each timepoint, and lack of BDNF measurements at baseline and at the time of behavior analysis in the SAL and CDDP groups we were unable to compare intra-group changes longitudinally or correlate our behavior findings with serum BDNF levels.

Pharmacological approaches to augment BDNF may provide a feasible strategy to prevent CRCI. To identify whether BDNF enhancement may protect against loss of dendritic spines and arborization in neurons, we conducted an *in vitro* screening of neuronal morphology in primary rat hippocampal neurons immunostained for PSD-95. We tested riluzole (FDA-approved for ALS) and two ampakines, CX546, CX1739, for their ability to protect neurons against cisplatin-induced reductions in dendritic spine density and arborization. All three agents prevented cisplatin-induced reductions in PSD-95 puncta density and dendritic arborization. Short-term exposure to BDNF, riluzole, or CX546, did not prevent cisplatin-induced reductions in NSC viability. Although our studies (Suppl. Fig. 3) showed that embryonic NSCs may have a narrow tolerance to riluzole (>5 μM) *in vitro*, riluzole does not impair NSC survival at clinically relevant doses (Hachem et al., 2015). The plasma concentration of riluzole in patients with ALS who received the clinically used dose of 50 mg bid, ranges between 0.9 and 1.6 μM (Lacomblez et al., 1996). Riluzole and CX546 exert anti-glutamatergic properties. While neurons are highly sensitive to glutamate excitotoxicity, glutamate enhances the proliferation and survival of embryonic NSCs at high concentrations, which may explain our findings showing that riluzole and CX546 do not increase NSC proliferation (Suppl. Fig. 3B, C) (Brazel et al., 2005). Screening of these compounds in two human ovarian cancer cell lines (OVCAR8 and SKOV3.ip1) revealed a minimal effect on cisplatin's anticancer efficacy.

The addition of *in vivo* immunohistochemical analyses of neurogenesis, and pre- and post-synaptic markers in our study would have enabled us to examine the long-term effects of cisplatin on neuronal health and synaptic plasticity. To address this limitation, future studies will assess the efficacy of BDNF enhancing agents to ameliorate cisplatin-induced CRCI, comparing behavioral findings to dendritic morphology following cisplatin completion. Although our studies focused on BDNF enhancement as a strategy for preventing cisplatin-induced neuronal damage, we cannot exclude the effects of cisplatin-induced reductions of BDNF on other cell types, including microglia and oligodendrocytes. Geraghty et al. have reported that methotrexate results in microglial-dependent reduction of BDNF expression, which impairs activity-dependent myelination (Geraghty et al., 2019). Cisplatin has been shown to reduce myelination (Chiu et al., 2018), and it also affects glutamate signaling in the CNS (Alhadeff et al., 2015). BDNF modulates synaptic activity by influencing the activity-dependent regulation of the structure and function of glutamatergic

synapses, thereby learning and memory. In turn, glutamate stimulates the transcription and production of BDNF in neurons and glial cells (Zafra et al., 1991). Glutamate, a major excitatory neurotransmitter in the CNS, acts through ionotropic glutamate receptors (iGluRs: NMDA, AMPA, kainate) and metabotropic glutamate receptors (mGluRs1–4). The role of glutamatergic signaling in the CNS is well established; however, glutamatergic signaling is also evident in peripheral tissues and cancer. Ionotropic GluRs and mGluRs subunits are expressed in a variety of non-CNS cancer cell lines and tumors, including colorectal (Chang et al., 2005), breast (Speyer et al., 2012), gastric (Liu et al., 2007), and prostate cancers (Abdul and Hoosein, 2005). Stepulak et al. compared the expression of AMPA (*GluR1–4*), NMDA (*NR1-NR3B*), kainite (*GluR5-GluR7, KA1, KA2*), and metabotropic (*mGluR1–8*) glutamate receptor subunits in various human cancer cell lines by RT-PCR and western blot to normal human brain tissue and found that all subunits were expressed in the cancer cell lines. Most analyzed subunits were expressed at lower levels than normal human brain tissue. Whole-cell current recordings from A549 (lung carcinoma) and TE671 cells (rhabdomyosarcoma/medulloblastoma) revealed that NMDA and AMPA receptors were functional; however, the evoked currents were small, which is consistent with the low expression of these receptor subunits in these cell lines compared to the brain (Stepulak et al., 2009). Given the structural heterogeneity of glutamate receptors, which results from distinct subunit isoforms following post-transcriptional modifications (RNA editing and alternative splicing), various glutamate receptor subtypes could also exist in non-CNS tumors. This, in addition to the varying expression of glutamate receptor subunits across different tumor types, suggests that potential neuroprotective compounds for CRCI that modulate glutamate signaling must be tested in cancer models. Riluzole has been shown to modulate glutamate-dependent and independent signaling by inhibiting glutamate release and reducing glutathione levels in pancreatic cancer cells and glioma (Roy et al., 2022; Khan et al., 2019). Indeed, riluzole has also been shown to have anti-cancer properties in multiple cancer cell lines and to synergize with paclitaxel in triple-negative breast cancer and cisplatin in colorectal cancer by decreasing cell proliferation and promoting apoptosis (Blyufer et al., 2021; Fortunato, 2017; Speyer et al., 2017).

Our studies provide evidence that reductions in BDNF are associated with cisplatin-induced loss of dendritic spines, arborization, and cognitive deficits. Although our studies support BDNF enhancement in ampakine and riluzole neuroprotection against cisplatin-induced CRCI, we cannot exclude modulation of glutamate signaling as a potential protective mechanism of these agents against CRCI. Future studies will involve testing these pharmacological strategies in clinically relevant, cancer-bearing models to determine the safety and efficacy of BDNF enhancement in preventing CRCI.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data availability

Data will be made available on request.

## Abbreviations

| | |
|---|---|
| **CDDP** | Cisplatin |
| **RZ** | Riluzole |
| **PSD-95** | Postsynaptic density-95 |
| **CRCI** | Cancer-Related Cognitive Impairments |
| **BDNF** | brain-derived neurotrophic factor |
| **SAL** | saline |
| **VEH** | vehicle |
| **NOR** | novel object recognition |
| **OFT** | open field test |

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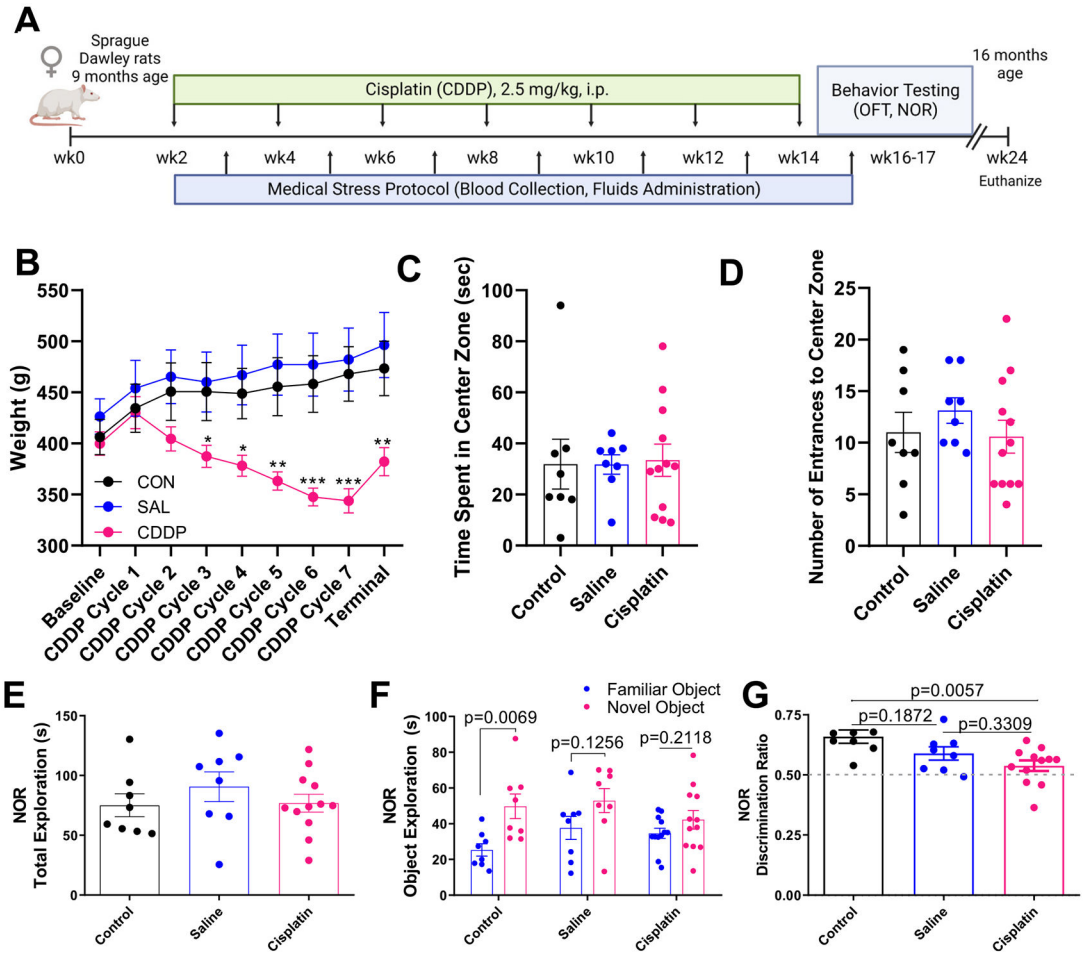


**Fig. 1.**

Chronic CDDP exposure and medical stress affect cognitive function and serum BDNF levels in a female middle-aged rat model. (A) Schematic representation of the experimental design. 9-month-old female retired-breeder Sprague Dawley rats were injected with vehicle (SAL, saline, $n = 8$) or cisplatin (CDDP, 2.5 mg/kg, i. p., $n = 12$) once every two weeks for 13 weeks. CDDP and SAL rats were subjected to the medical stress protocol, which included hydration therapy (5–10 mL 0.9% saline/day, s.q.) for five consecutive days during CDDP treatment and lateral tail vein blood collections 72 h after each CDDP dose. Cage mates that did not receive CDDP/SAL injections nor blood collections served as healthy age-matched controls (CON, $n = 8$) for this study. Fourteen weeks after the start of CDDP treatment, all rats were assessed for anxiogenic behavior using the open field recognition test (OFT) and cognitive function using the novel object recognition task (NOR). Rats were euthanized ten weeks following the completion of CDDP, and serum was collected for BDNF analyses. (B) Effect of CDDP regimen on body weight (grams) over time. Quantification of (C) time spent in the center zone and (D) number of entrances to the center zone of the arena over 10 min in the OFT. (E) Quantification of total time spent exploring novel and familiar objects during NOR. (F) Medical stress and CDDP treatment impaired cognitive function as SAL and CDDP rats had a reduced preference for the novel object compared to the familiar object during NOR. (G) CDDP-exposed rats had a significantly

lower discrimination ratio [(novel object exploration (sec))/(total object exploration (sec))] compared to healthy age-matched controls (CON). Data are shown as mean ± SEM; each point represents one rat (C-G). Not significant = ns, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$, as determined by one-way ANOVA with Tukey's *post hoc* analysis for multiple comparisons test. Figure prepared with BioRender.

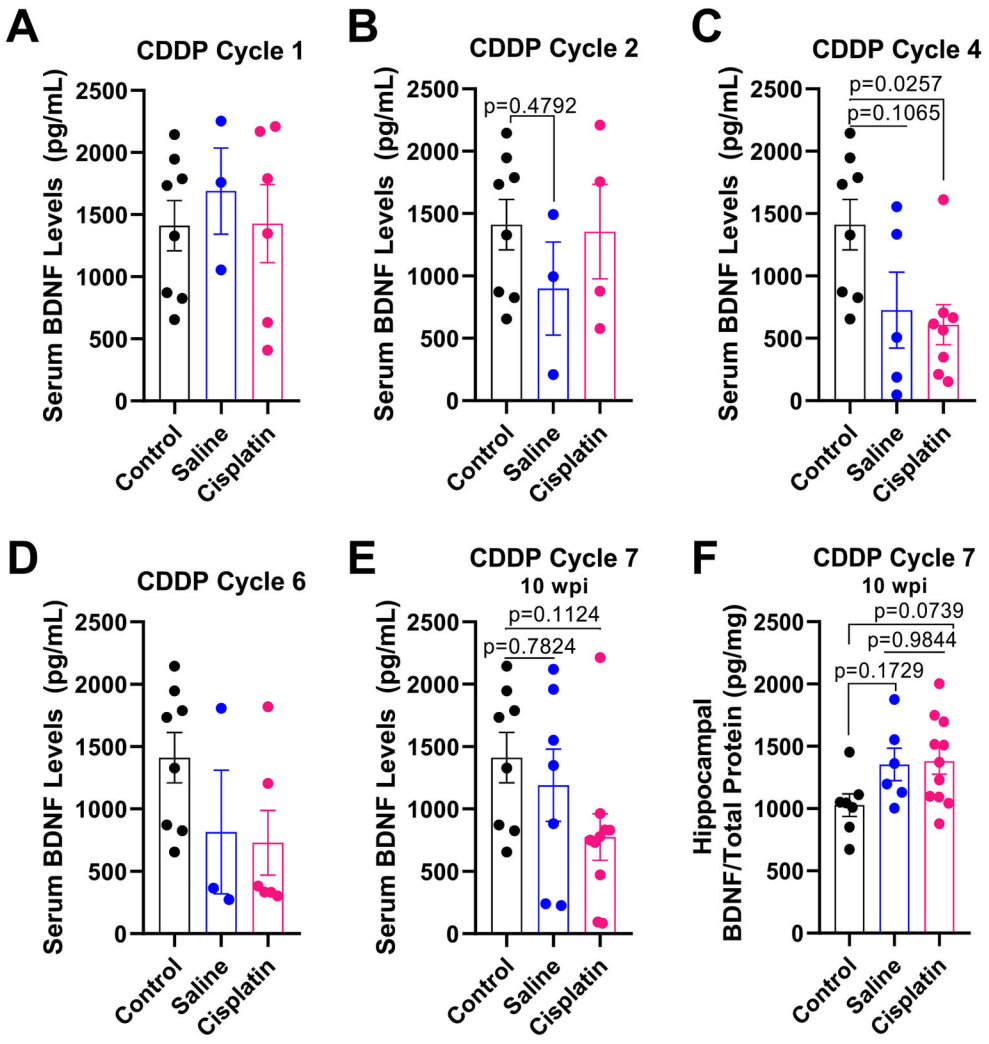


**Fig. 2.**

Longitudinal BDNF measurements in female middle-aged rat model following chronic CDDP exposure and medical stress. ELISA-based quantification of BDNF levels from rat serum showed longitudinal and dose-dependent changes in SAL and CDDP groups. Serum BDNF levels were quantified 72 h following: (A) CDDP Cycle 1 (2.5 mg/kg, i.p.), (B) CDDP Cycle 2 (cumulative 5 mg/kg, i.p.), (C) CDDP Cycle 4 (cumulative 10 mg/kg, i.p.), (D) CDDP Cycle 6 (cumulative 15 mg/kg, i.p.), and (E) 10 weeks following CDDP Cycle 7 (cumulative 17.5 mg/kg, i.p.). (F) Hippocampal BDNF levels were also measured 10 weeks following CDDP Cycle 7. Data are shown as mean ± SEM; each point represents one rat (A-F). Not significant = ns, $*P < 0.05$, $**P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, as determined by one-way ANOVA with Tukey's *post hoc* analysis for multiple comparisons test.

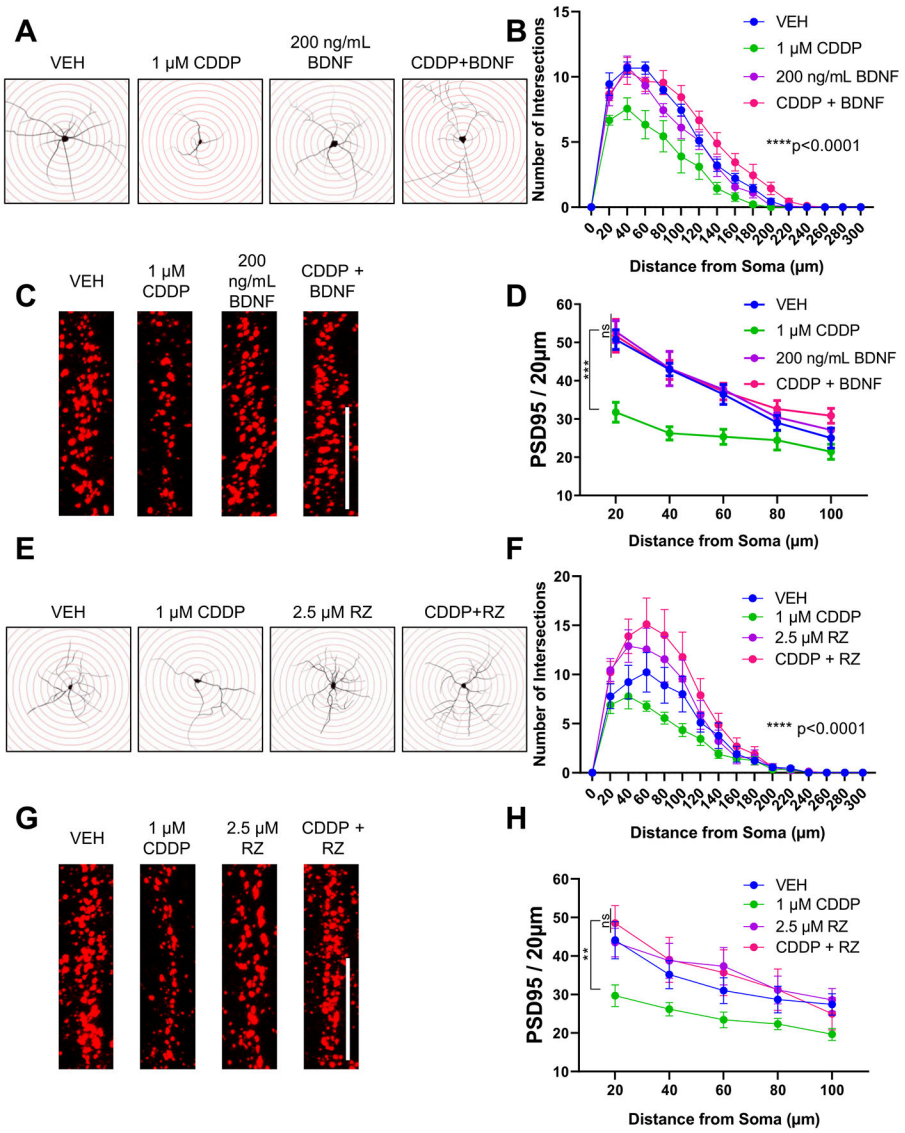


**Fig. 3.**

*In vitro*, BDNF augmentation is protective against cisplatin-induced loss of dendritic arborization and PSD-95. (A) Mature rat hippocampal neurons were immunostained for PSD-95 following exposure to 1 μM CDDP with or without 200 ng/mL BDNF co-treatment for 24 h. Representative images of neurons reconstructed with Adobe Illustrator and superimposed over concentric Sholl circles (20 μm increments). (B) Quantification of intersection points between the concentric circles and dendritic branches at increasing distance from the soma. (C) Representative images and (D) quantification of PSD-95 density at increasing distance from the soma in neurons exposed to 1 μM CDDP with or without co-treatment of 200 ng/mL BDNF for 24 h. (E) Neurons were pre-treated with 2.5 μM RZ for 1 h, followed by exposure to 1 μM CDDP for 24 h. Representative images of reconstructed neurons superimposed over Sholl circles and (F) quantification of dendritic branch points using Sholl analysis. (G) Representative images and (H) quantification of PSD-95 density in neurons pre-treated with 2.5 μM RZ for 1 h followed by exposure to 1

μM CDDP for 24 h. Data are shown as mean ± SEM; (B, F) $n = 9$ neurons/group; (D, H) $n = 6$ neurons/group, 2 dendritic branches/neuron. Not significant = ns, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, as determined by two-way repeated measures ANOVA with Tukey's *post hoc* analysis for multiple comparisons test. Scale bars = 10 μm.

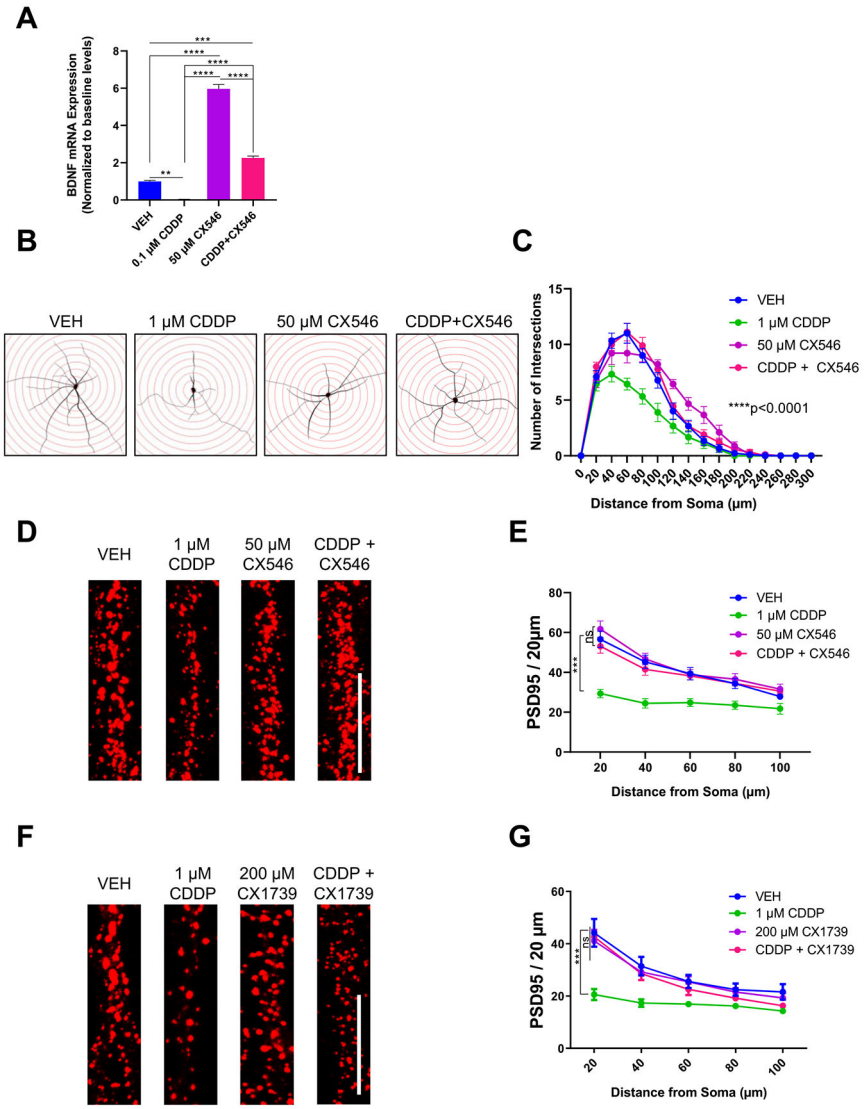


**Fig. 4.**

*In vitro*, ampakine CX546 prevents cisplatin-induced loss of BDNF expression, dendritic arborization, and PSD-95. (A) Cisplatin significantly reduced BDNF mRNA after exposure to 1 μM cisplatin at 24 h, which was prevented by co-treatment with 50 μM CX546. (B) Representative images of reconstructed neurons superimposed over 20 μm concentric Sholl circles, and (C) Sholl analysis quantification of mature hippocampal neurons exposed to 1 μM cisplatin with or without co-treatment of 50 μM CX546 for 24 h. (D) Representative images of dendritic branches immunostained for PSD-95 and (E) quantification of PSD-95 puncta density along distance from soma following exposure to 1 μM CDDP with or without 50 μM CX546 for 24 h. (F) Representative images and (G) quantification of PSD-95 puncta along distance from soma following 48 h co-treatment with 1 μM cisplatin and with or without 200 μM ampakine CX1739. (C) $n = 9$ neurons/group; (E, G) $n = 6$ neurons/group, 2 dendritic branches/neuron. Data are shown as mean ± SEM; not significant = ns, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ as determined by two-way repeated measures ANOVA with Tukey's *post hoc* analysis for multiple comparisons test. Scale bars = 10 μm.

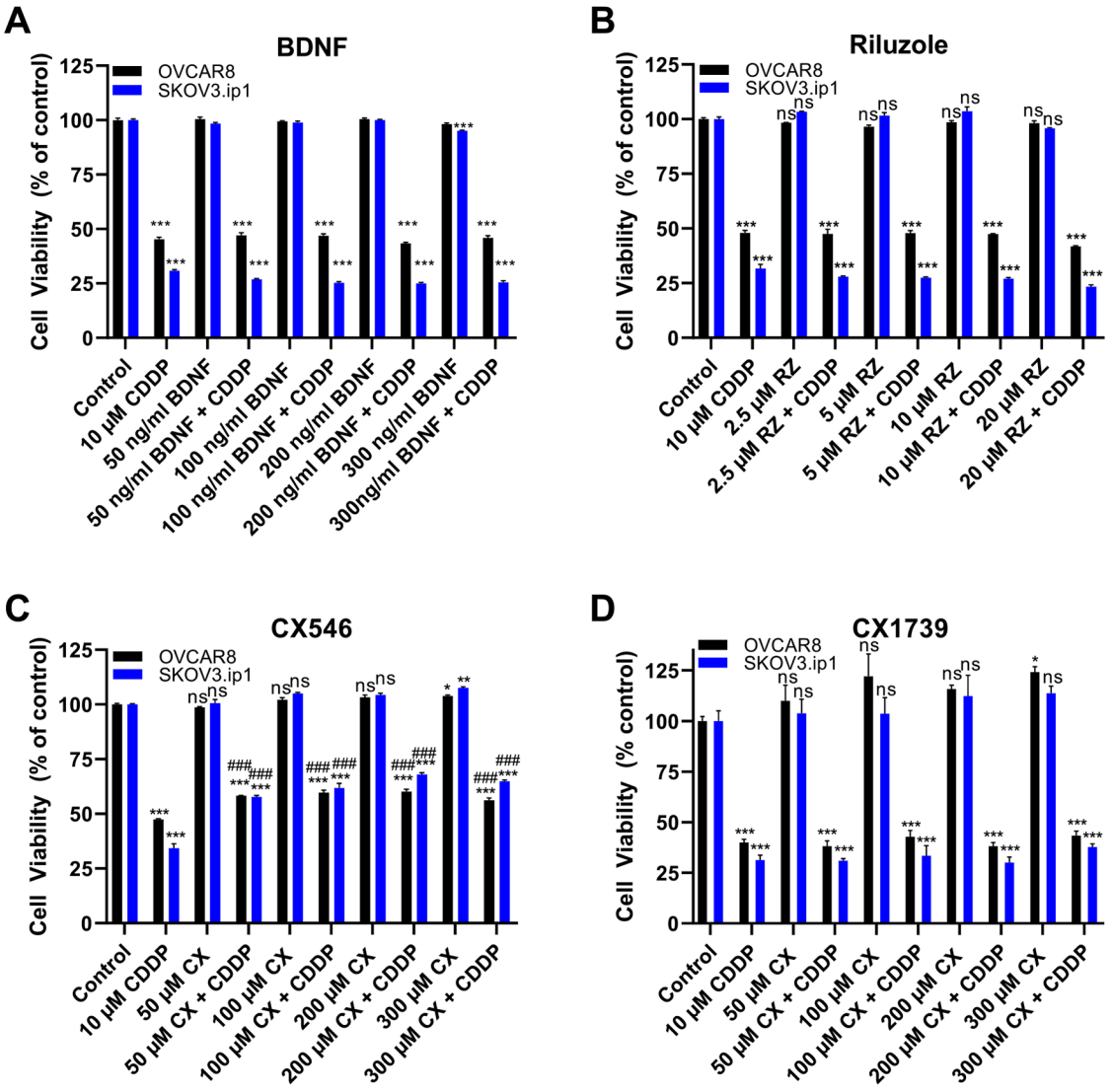


**Fig. 5.**

*In vitro*, screening of BDNF-enhancing pharmacological agents riluzole and CX546, and non-BDNF enhancing CX1739 in human ovarian cancer lines OVCAR8 and SKOV3.ip. OVCAR8 and SKOV3.ip1 were plated at 10,000 cells/well on 96-well plates and treated with 10 μM CDDP with or without graded doses of (A) BDNF (50 ng/mL, 100 ng/mL, 200 ng/mL, 300 ng/mL), (B) riluzole (2.5 μM, 5 μM, 10 μM, 20 μM), (C) CX546 (50 μM, 100 μM, 200 μM, 300 μM), and (D) CX1739 (50 μM, 100 μM, 200 μM, 300 μM) for 72 h. Cell viability was normalized to vehicle control. (A-D) $n = 3$ wells/group. Data are shown as mean ± SEM; not significant = ns, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, denotes statistical significance compared to vehicle control. $###P < 0.001$ denotes statistical significance compared to 10 μM CDDP. Statistical significance was determined by one-way ANOVA with Tukey's *post hoc* analysis for multiple comparisons test.