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ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/iett20

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To cite this article: Dasiel Borroto-Escuela, Pedro Serrano-Castro, Jose Andrés Sánchez-Pérez, Miguel Angel Barbancho-Fernández, Kjell Fuxe & Manuel Narváez (2024) Enhanced neuronal survival and BDNF elevation via long-term co-activation of galanin 2 (GALR2) and neuropeptide Y1 receptors (NPY1R): potential therapeutic targets for major depressive disorder, Expert Opinion on Therapeutic Targets, 28:4, 295-308, DOI: 10.1080/14728222.2024.2342517

To link to this article: https://doi.org/10.1080/14728222.2024.2342517

EXPERT OPINION ON THERAPEUTIC TARGETS
2024, VOL. 28, NO. 4, 295–308
https://doi.org/10.1080/14728222.2024.2342517

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ORIGINAL RESEARCH

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Enhanced neuronal survival and BDNF elevation via long-term co-activation of galanin 2 (GALR2) and neuropeptide Y1 receptors (NPY1R): potential therapeutic targets for major depressive disorder

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ABSTRACT

Background: Major Depressive Disorder (MDD) is a prevalent and debilitating condition, necessitating novel therapeutic strategies due to the limited efficacy and adverse effects of current treatments. We explored how galanin receptor 2 (GALR2) and Neuropeptide Y1 Receptor (NPYY1R) agonists, working together, can boost brain cell growth and increase antidepressant-like effects in rats. This suggests new ways to treat Major Depressive Disorder (MDD).

Research Design and Methods: In a controlled laboratory setting, adult naive Sprague-Dawley rats were administered directly into the brain's ventricles, a method known as intracerebroventricular (ICV) administration, with GALR2 agonist (M1145), NPYY1R agonist, both, or in combination with a GALR2 antagonist (M871). Main outcome measures included long-term neuronal survival, differentiation, and behavioral.

Results: Co-administration of M1145 and NPYY1R agonist significantly enhanced neuronal survival and maturation in the ventral dentate gyrus, with a notable increase in Brain-Derived Neurotrophic Factor (BDNF) expression. This neurogenic effect was associated with an antidepressant-like effect, an outcome partially reversed by M871.

Conclusions: GALR2 and NPYY1R agonists jointly promote hippocampal neurogenesis and exert antidepressant-like effects in rats without adverse outcomes, highlighting their therapeutic potential for MDD. The study's reliance on an animal model and intracerebroventricular delivery warrants further clinical exploration to confirm these promising results.

ARTICLE HISTORY
Received 1 March 2024
Accepted 5 April 2024

KEYWORDS
Antidepressant effects; galanin receptor 2 (GALR2) agonist; major depressive disorder (MDD); neuropeptide Y1 receptor (NPYY1R) agonist; neurogenesis; rodent model

1. Introduction

Depression, a common mental health issue, arises from complex interactions among biological, psychological, and environmental factors [1–3]. Affecting upwards of 300 million people worldwide, it constitutes about 4.4% of the global populace, as indicated by the World Health Organization and corroborated by the Global Burden of Disease Study [4,5]. The underlying biological processes contributing to depression include, but are not limited to, alterations in monoamine levels, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, changes in synaptic structures, inflammation, and neurogenesis, with hippocampal neurogenesis being particularly significant [6].

The hippocampus, a critical area of the brain for learning, memory, and mood regulation, often appears smaller in people with depression, as shown by MRI (Magnetic Resonance Imaging) scans [7–12]. This atrophy is mirrored at the cellular level, where reductions in mature granule cells and the granular layer's volume have been observed in MDD patients [13]. The dentate gyrus (DG), central to neurogenesis, is crucial for integrating new neurons into existing circuits, supporting cognitive functions and stress resilience – a process compromised in depression, potentially exacerbating the disorder [14–22]. Notably, the ventral DG's response to emotional stimuli and its role in stress modulation highlight the therapeutic potential of targeting neurogenesis. Studies have shown that antidepressants can stimulate neural progenitor proliferation in the DG, offering a pathway to mitigate depression by enhancing hippocampal neurogenesis [23–34].

However, the efficacy of antidepressants targeting monoaminergic systems remains limited, prompting the exploration of alternative therapeutic approaches given the high failure

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14728222.2024.2342517

rate of new antidepressant drugs in clinical trials [35]. Emerging research has begun to pivot from monoamine-based models to investigating the roles of neuropeptides like neuropeptide Y (NPY) and neurotrophic factors such as Brain-Derived Neurotrophic Factor (BDNF) in mood regulation [34,36–38]. Neuropeptide Y (NPY), a peptide containing 36 amino acid residues with a broad distribution in the brain, plays multifaceted roles in emotional regulation, neuroprotection, and stress modulation, particularly within key regions such as the amygdala and hippocampus [39–42]. Its significance in mood disorders is evidenced by altered NPY1 receptor (NPY1R) levels in depression models and its capacity to mitigate depressive behaviors and enhance BDNF levels, indicating a crucial role in mood regulation and neurogenesis [43–48]. These findings highlight the potential of targeting NPY and NPY1R pathways for developing novel antidepressant strategies.

Galanin (GAL), akin to Neuropeptide Y, plays a significant role in the central nervous system, influencing mood regulation through its receptors distributed in brain regions like the ventral CA1 and the dentate gyrus (DG) [49–51]. The activation of GAL2 receptors (GALR2), in particular, has been associated with antidepressant-like effects in experimental models, underscoring GAL's therapeutic potential for mood disorders. Experimental evidence suggests that GAL's modulation of its receptors can influence depressive behaviors, with GALR2 activation linked to positive antidepressant outcomes and GalR2-knockout models displaying depression-consistent behaviors. Recent developments, including a GALR2 agonist, have further highlighted GAL's promise in depression therapy [52–57].

Emerging clinical evidence indicates that alterations in NPY and GAL receptors are associated with depression and suicidal behavior, underlining the therapeutic potential of targeting these neuropeptide systems in the context of stress-induced mood disorders and emphasizing the significance of exploring the NPY-GAL interaction for novel depression treatments [58,59]. Building upon these insights, extensive research has explored the intricate interplay between NPY and GAL, particularly focusing on the potential formation of NPY1R-GALR2 heteroreceptor complexes in several brain regions, including the amygdala, hippocampus, hypothalamic areas, and the medial prefrontal cortex [60–66]. The intricate interplay between the signaling pathways of NPY and GAL, and the possibility of these neuropeptides forming heteroreceptor complexes, offers a groundbreaking avenue for addressing the complex nature of depression. By exploring the synergistic effects of NPY1R and GALR2 agonists, our research aims to unveil novel mechanisms of action for antidepressant therapies, potentially overcoming the drawbacks of existing treatments. This focus not only highlights the innovative approach of our study but also emphasizes its potential to contribute significantly to the development of targeted, more effective antidepressant strategies.

Building upon the recognition of the limited efficacy and adverse effects associated with current antidepressants, this study seeks to explore novel therapeutic strategies for Major Depressive Disorder (MDD). Specifically, our aim is to investigate the synergistic effects of NPY1R and GALR2 agonists on hippocampal neurogenesis and their influence on antidepressant-like effects in an experimental model. By focusing on these neuropeptide receptors' roles within the ventral hippocampus, we propose a potentially groundbreaking approach to addressing the complexities of MDD. This study does not only aim to enhance our understanding of the neurobiological bases of depression but also to identify innovative targets for the development of antidepressant therapies. Ultimately, our research attempts to contribute to the advancement of treatments capable of more effectively managing the multifaceted symptoms of depression, potentially reducing the reliance on current treatments with known limitations and side effects.

2. Materials and methods

2.1. Animal housing, care, and experimental design

Male Sprague-Dawley rats, sourced from CRIFFA, Barcelona, weighing between 200 and 250 grams and aged 6–8 weeks, were housed in large group cages, accommodating six animals each, to promote social interaction and environmental enrichment. To enhance their environmental complexity and stimulate natural behaviors, each cage was supplemented with paper shavings and cardboard tubes, allowing the rats to burrow and explore. These enrichments were designed to improve the well-being and physiological robustness of the animals, potentially reducing stress-induced variability in experimental outcomes. The housing conditions maintained a strict 12-hour light/dark cycle, with environmental parameters such as relative humidity (55–60%) and temperature (22 ± 2°C) carefully controlled. Behavioral experiments were conducted between 09:00 and 14:00 hours. All animal care and experimental protocols were conducted in strict adherence to the EU Directive 2010/63/EU and Spanish legislation (Real Decreto 53/2013), with approval from the University of Málaga's Local Committee for the Ethics of Animal Experimentation, Spain (CEUMA 45–2022-A).

Animals were randomly assigned to their respective experimental groups using the drawing lots method. This randomization process was crucial for minimizing selection bias and ensuring the integrity of our experimental design. By randomly allocating animals to each group, we aimed to evenly distribute any inherent biological variability among the experimental conditions, thus enhancing the reliability and validity of our findings.

To ensure unbiased observation and analysis, blinding was implemented during the assessment phase. Individuals analyzing the data were not involved in the experimental procedures and were unaware of the group allocations. Furthermore, the names of the animal groups were coded, concealing their identity from the analysts. This blinding strategy was crucial for eliminating observer bias, allowing for objective assessment and interpretation of the behavioral and physiological outcomes.

2.2. Preparation of drugs

Peptides were freshly prepared by dilution in artificial cerebrospinal fluid (aCSF), composed of 120 mM NaCl, 20 mM

NaH2CO3, 2 mM KCl, 0.5 mM KH2PO4, 1.2 mM CaCl2, 1.8 mM MgCl2, 0.5 mM Na2SO4, and 5.8 mM D-glucose, adjusted to a pH of 7.4. This solution served as the vehicle for control groups. Receptor-specific agonists and antagonists, including Galanin receptor 2 agonist (M1145), Y1R receptor agonist [Leu31, Pro34] NPY, and GALR2 Antagonist M871, were procured from Tocris Bioscience (Bristol, UK). Detailed methodologies for the intracerebroventricular (icv) administration of these peptides are elaborated in the Supplementary Material.

2.3. Experimental design

The experimental subjects were categorized into five groups: (1) control (aCSF), (2) treated with M1145 (3 nmol), (3) treated with [Leu31, Pro34] NPY (3 nmol), (4) a combination treatment of M1145 and Y1R agonist, and (5) a trio treatment involving M1145, [Leu31, Pro34]NPY, and the GALR2 antagonist M871 (3 nmol each), with four rats in each group. The treatment was administered once daily over a three-day period, aligning with established protocols that suggest the durability of NPY's efficacy [46,67–71]. Behavioral and neurochemical assessments were conducted on separate sets of animals 3 weeks after the peptide administration. This timeline aligns with findings from Decressac's study on the neurogenic effects of NPY1R [69], along with other research, demonstrating that newborn neurons can start to functionally contribute to hippocampal activity in rats within 2–3 weeks [15,17,18].

2.4. Evaluation of hippocampal cell survival

Rats received two intraperitoneal (i.p.) injections of 5′-Bromo-2′-deoxyuridine (BrdU, Sigma, St. Louis, MO, U.S.A.) dissolved in a sterile 0.9% NaCl solution at a concentration of 15 mg/mL, at a dosage of 50 mg/kg body weight every 2 hours over 3 days, starting at 9:00 AM. Three weeks post-icv treatment, animals were anesthetized with pentobarbital (Mebumal, 100 mg/kg, i.p.) and perfused with 4% paraformaldehyde. Brain tissues were sectioned at 30 µm through the ventral hippocampus, following protocols established in previous studies [15,69,72,73].

2.4.1. Immunohistochemistry

Brain sections were incubated free-floating in saline sodium citrate buffer (pH 6; 10 nM sodium citrate) for 90 min at 65°C, followed by 30 min with 0,6% H2O2 to remove endogenous peroxidases. After 30 min in 2 M hydrochloric acid (HCl) to denature deoxyribonucleic acid (DNA), sections were incubated for neutralization with 0.1 M sodium borate (pH 8). Then, slices were incubated at 4◦C overnight with a primary antibody against BrdU (Abcam, ab152095, 1:1000) in 2,5% donkey serum. Following additional washed with PBS and incubated with a secondary antibody for 90 min (biotinylated anti-rabbit IgG, 1/200, Vector Laboratories), sections were amplified with ExtrAvidin peroxidase (Sigma, St. Louis, MO, U.S.A.) diluted 1:100 in darkness at room temperature for 1 hour. Immunolabeling was exposed with 0.05% diaminobenzidine (DAB; Sigma) and 0.03% H_2O_2 in PBS. After various washes, sections were mounted on gelatin-coated slides, dehydrated in graded alcohols, and cover-slipped in DePeX mounting medium (Merck Life Science SLU, Darmstadt, Germany). BrdU-labeled cells with morphological characteristics of glial precursors, i.e. small, irregularly shaped cell bodies, were excluded. Only round, regularly shaped BrdU-positive nuclei located in the DG were counted since new granule cells migrate approximately 2 cell body widths from the SGZ into the granule layer [46]. As previously described, BrdU-labeled cells were analyzed using the optical fractionator method in unbiased stereological microscopy (Olympus B×51Microscope, Olympus, Denmark) [62,63,65] (see Supplementary Material for details).

2.4.2. Double immunofluorescence

Following established protocols, sections were blocked, permeabilized, and incubated with primary antibodies against BrdU and either DCX or NeuN [60,61,63–65]. Briefly, an initial incubation with blocking (5% goat serum) and permeabilization (0.3% triton X100 in PBS) solutions was performed for 60 min each. Pair of primary antibodies rabbit anti-BrdU (Abcam, ab152095, 1:1000)/mouse anti-DCX (C-18, Santa Cruz, 1:500) or rabbit anti-BrdU (Abcam, ab152095, 1:1000)/mouse anti-NeuN (Abcam, ab1042241, 1:1000) were used to incubate the sections for 24 h, at 4°C. Then, incubations were performed with proper secondary antibodies: Donkey anti-mouse AlexaFluor 488 (Abcam, ab150105, 1:200) and Donkey anti-rabbit AlexaFluor 647 (Abcam, ab150075, 1:200). Sections were mounted on slides with a fluorescent mounting medium with 4,′6-diamidino-2-phenylindole (DAPI) to detect nuclei (Abcam, ab104139). BRDU/DCX- and BRDU/NeuN double-stained cells in the DG were quantified using z-scan confocal microscopy (Leica Stellaris 8) at 40X magnification. The entire length of the DG was assessed through the septo-temporal axis of the hippocampus, analyzing at least four representative 150 µm, evenly spaced sections per animal. All analyzes were conducted in sequential scanning mode to eliminate potential cross-bleeding between channels. Each cell was scrutinized using a multi-channel configuration, and colocalization with NeuN or GFAP was verified by examining multiple optical planes for each cell on the z-axis. Z-stacks were generated at 0.85 µm intervals throughout the 30 µm section to ensure accurate double-labeling of BrdU-IR cells [63,74].

2.5. Evaluation of brain-derived neurotrophic factor (BDNF) induction in the ventral dentate gyrus

Various free-floating sections were processed for antigen retrieval at 65°C for 90 minutes in saline sodium citrate buffer (pH 6; 10 nM sodium citrate), followed by a 30-minute treatment in 0.6% H2O2. Subsequently, slices were incubated overnight at room temperature with primary antibody rabbit anti-BDNF (Chemicon, Sigma-Aldrich, AB1534SP, 1:500) in 2.5% donkey serum. Post-PBS washes, slices were treated with biotinylated anti-rabbit IgG (1:200, B8895, Sigma, St. Louis, MO, U.S.A.) for 90 minutes and then with ExtrAvidin peroxidase (1:100, Sigma, St. Louis, MO, U.S.A.) in darkness for 1 hour. Detection was completed with 0.05% diaminobenzidine (DAB) and 0.03% H2O2 in PBS, followed by mounting on gelatin-coated slides and coverslipping with DePeX mounting medium (Merck Life Science SLU, Darmstadt, Germany). The BDNF-marked cells were then analyzed via optical fractionator methodology in unbiased stereological microscopy (Olympus B×51Microscope, Olympus, Denmark).

2.6. Hippocampal cell culture and conditions

Rat primary hippocampal neuronal cells were purchased from QBM Cell Science (Montreal, Canada) and cultured in Neuro basal medium supplemented with 10% FBS, 2 mM GlutaMAX-1, 1 mM sodium pyruvate, 100 U/ml penicillin G, and 100 µg/ml streptomycin and 2% B-27 supplement at 37°C in a humidified 10% CO_2 environment according to manufacturer's instructions. Half part of the medium was changed every 3 days. The cells were grown under the above conditions (control condition) for 7 days. Cultured hippocampal neurons were grown and treated for 14 days under specific pharmacologic conditions. Treated hippocampal cells were divided into experimental groups: (1) Control group; (2) M1145-treated group (100 nM); (3) Y1R agonist-treated group receiving an NPYY1R agonist [Leu^{31},Pro^{34}]NPY (100 nM); (4) GAL+Y1R: Group treated with both substances; (5) GAL+Y1 +M871: Group incubated with GAL, [Leu^{31},Pro^{34}]NPY and the GALR2 antagonist (M871; 1 µM). Cells were grown on poly-D-lysine-coated glass coverslips and fixed with 4% formaldehyde solution for 20 min followed by two washes with PBS containing 20 mM glycine to quench the aldehyde groups.

2.7. Hippocampal neuronal cells' viability analysis

The MTS assay determined cell viability in rat primary hippocampal neuronal cells using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (G3580, Promega, Madison, WI, U.S.A.). This assay determines the levels of cellular 3-[4,5-dimethylthiazol-2-yl-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2 H tetrazolium, inner salt] (MTS) reduction to formazan, a measure of mitochondrial function. Cultured hippocampal neurons (7 days in vitro) were collected, and approximately 2×10^4 cells per well were plated into 96-well plates, which were treated for 0, 3, 6, and 9 days, respectively in B27-deprived medium, and then 20 µL of MTS solution was added to each well. Plates were incubated at 37°C for 3 h. The absorbance was determined with a POLARstar Optima plate reader (BMG Labtech) at 490 nm, directly proportional to the number of living cells in the culture. This protocol was performed as described in [63,75].

2.8. Analysis of neurite length

A different set of cultured hippocampal neurons was grown under the specific pharmacologic conditions described above. Cells were grown on poly-D-lysine-coated glass coverslips and fixed with 4% formaldehyde solution for 20 min followed by two washes with PBS containing 20 mM glycine to quench the aldehyde groups. Then, after permeabilization with PBS containing 0.2% Triton X-100 for 5 min, cells were treated with PBS containing 1% bovine serum albumin. Cells were labeled with Neuro-Chrom Pan Neuronal Marker primary antibody (ABN2300, 1:100, Sigma-Aldrich; Merck Life Science S.L.U.) for 1 h, extensively washed, and stained with the green fluorescence secondary antibody goat anti-rabbit DyLight 488 (Jackson Laboratories InmunoResearch, 1:100). Cell nuclei were counterstained with DAPI (blue) contained in the mounting medium. Acquisition of microscopy images and morphometric quantifications were performed as described [76,77].

2.9. Behavioral assessment of antidepressant-like effects

The antidepressant-like effect elicited by the administration of the Forced Swim Test (FST), a behavioral test for assessing despair-like behavior in rodents, often used in antidepressant screening [78–80].

Behavioral experiments were conducted between 09:00 and 14:00 hours, three-week post-administration. Naive rats were acclimatized to handling and the experimental environment before being randomly assigned to the experimental groups. The Open-Field Test (OFT) was used to determine eligibility for the FST. Rats were individually placed and allowed to freely explore the behavioral apparatus over a 10 minutes period. Locomotor parameter of distance moved was analyzed using the video tracking software Smart2.5 (Panlab, SL). After each trial, all surfaces were cleaned with 70% ethanol solution [72,81–83]. In the current study, the OFT was conducted followed by the Forced Swim Test (FST) on two consecutive days [72,81,82]. To address potential confounding variables associated with the sensitization procedure, the animals were subjected to the Forced Swim Test (FST) a single time [84]. The FST involved a 10-minute swim in a cylinder filled with water at 25 ± 0.2°C. Immobility, swimming and climbing behaviors were recorded and analyzed using Raton Time 1.0 software (Fixma S.L., Valencia, Spain). Immobility time was recorded when the animal made minimum movements necessary to keep its body afloat. Swimming time was regarded as the time when an animal performed an active swimming activity beyond the necessary movements to keep itself floating, while climbing was defined as vigorous forepaw movements directed toward the walls of the cylinder. Depression-related behavior was inferred from an increase in the time the rat spent immobile, which is thought to represent a lack of motivation to escape from the water. Post-test, rats were dried and returned to their home cages. Observers blinded to the experimental conditions conducted the behavioral experiments.

2.10. Statistical analysis

Data were analyzed with the assumption that each individual rat served as an experimental unit. Data were exhibited as mean ± SEM, with the number of samples denoted in the figure legends. GraphPad PRISM 8.0 (GraphPad Software, La Jolla, CA, U.S.A.) was harnessed for data examination. One-way ANOVA was conducted followed by the Newman-Keuls posttest for comparative analysis, or Student's unpaired t-test where necessary. Significance thresholds were defined as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

3. Results

3.1. GALR2 and Y1R agonist co-injection promotes survival of newborn neurons in the ventral hippocampus

Our investigation into the cellular mechanisms behind GALR2 and Y1R agonist icv subacute co-administration on adult ventral hippocampal cell proliferation was performed using the thymidine analogue 5-Bromo-2'-deoxyuridine (BrdU) (Figure 1(a)).

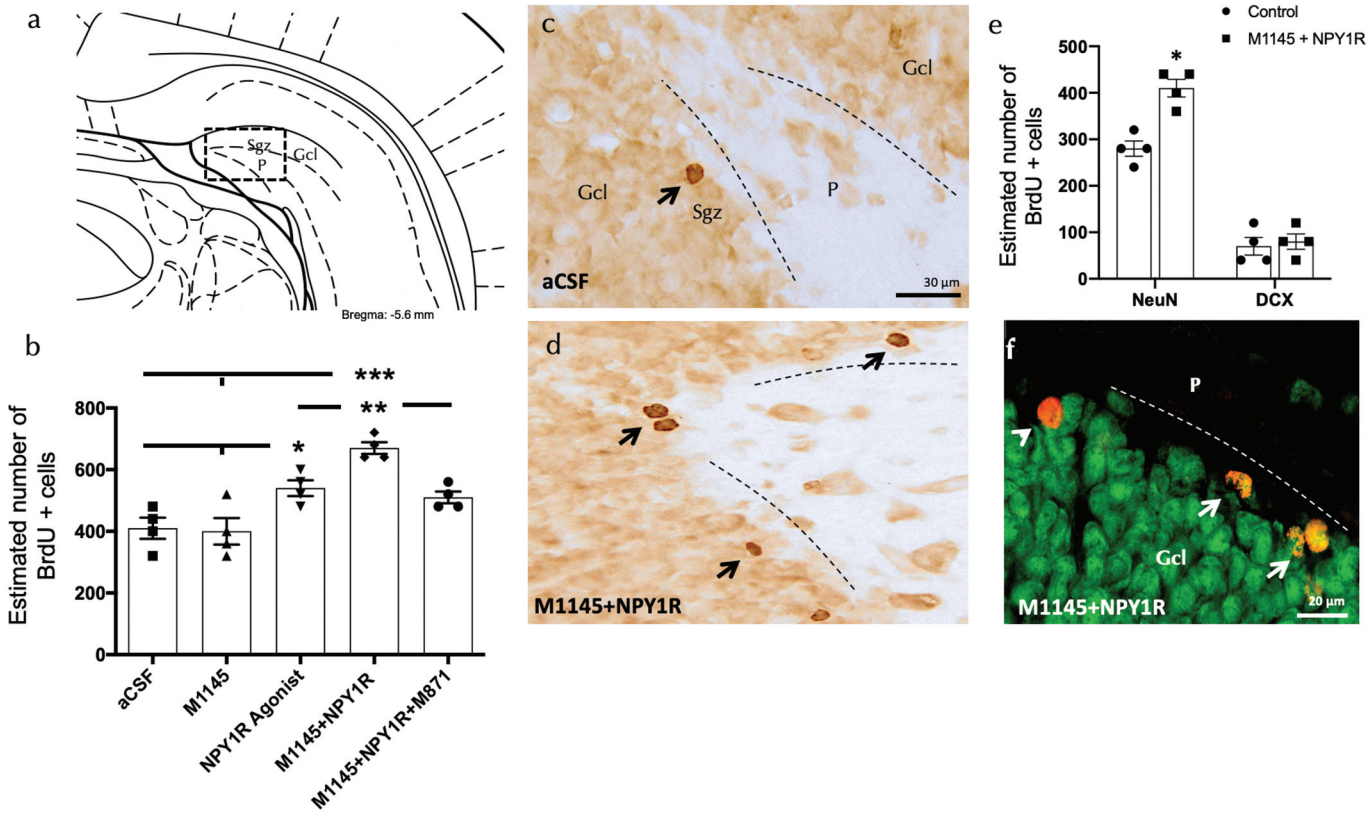

Figure 1. Cell survival analysis in the ventral dentate gyrus following intracerebroventricular co-administration of galanin receptor 2 and NPY1R.

Increase in neuronal survival within the ventral dentate gyrus following the intracerebroventricular (icv) co-administration of Galanin Receptor 2 (GALR2) and Neuropeptide Y1 Receptor (NPY1R) agonists in adult rats. Through the use of 5-Bromo-2′-deoxyuridine (BrdU) immunolabeling, this figure illustrates the effects of administering a GALR2 agonist (M1145) and an NPY1R agonist ([Leu31, Pro34]NPY), both individually and in combination, with and without the addition of a GALR2 receptor antagonist (M871). Panel (a) and (d) show the localization of BrdU-positive cells predominantly within the granule cell layer (Gcl) of the dentate gyrus in the ventral hippocampus, marked by round and regularly shaped BrdU-positive nuclei, referencing the Bregma coordinate -5.6 mm according to the stereotaxic atlas by Paxinos and Watson [85]. Panel (b) offers a quantitative analysis of the total number of BrdU-immunoreactive (IR) cells within the ventral hippocampus's dentate gyrus following the administration of control (aCSF), M1145, [Leu31-Pro34]NPY (NPY1R agonist), or their co-administration with or without M871, demonstrating statistically significant differences as indicated by $^{*}p < 0.05$ NPY1R agonist versus aCSF and M1145; $^{**}p < 0.01$ M1145+NPY1R versus Y1R agonist and M1145+NPY1R+M871; $^{***}p < 0.001$ M1145+NPY1R versus aCSF and M1145 ($n = 4$). Panel (d) highlights the marked increase in BrdU immunolabeling within the Gcl upon co-administration of M1145 and Y1R agonist compared to the control group (c), with arrows pointing out BrdU-positive neurons and dashed lines demarcating the Gcl of the dentate gyrus. Panel (e) quantifies BrdU-IR cells that are double-labeled with NeuN or DCX, revealing that the Y1R-GALR2 combination specifically promotes neuronal maturation, with data shown as mean ± SEM and $^{*}p < 0.05$ M1145+NPY1R versus control based on Student's unpaired t-test. Lastly, panel (f) displays a representative photomicrograph indicating BrdU+/NeuN+ cells (white arrows) alongside BrdU-/NeuN+ cells (white arrowheads) in the group treated with M1145 and Y1R agonist. The abbreviations used include: aCSF for control (artificial cerebrospinal fluid), M1145 for the Galanin receptor 2 agonist (3 nmol), NPY1R agonist for the NPY1R receptor agonist [Leu31, Pro34]NPY (3 nmol), M1145 + NPY1R for the co-administration of M1145 and Y1R agonist, and M1145 + NPY1R + M871 for the co-administration of M1145, Y1R, and GALR2 antagonist M871 (3 nmol).

ICV co-administration of M1145 and the Y1R agonist significantly increased the number of BrdU-IR profiles in the subgranular zone (Sgz) of the dentate gyrus compared to the control group, as well as the M1145 and Y1R agonist alone groups (one-way ANOVA, $F4,15 = 13.68$, $p < 0.001$), with post-hoc tests indicating significant differences compared to control, M1145, and Y1R agonist groups ($p < 0.001$ against control and M1145; $p < 0.01$ against Y1R agonist) (Figure 1(a,b,d)). The addition of GALR2 antagonist M871 blocked this effect ($p < 0.01$ in post-hoc test), indicating the participation of GALR2 in the M1145/NPYY1R agonist interaction to stimulate cell survival. Figure 1 shows the increase in BrdU-positive cells in the subgranular zone, highlighting the increased survival rate three weeks after the treatments.

Moreover, the icv administration of the Y1R agonist alone induced an increase in the number of BrdU positive cells in the subgranular zone (Sgz) of the ventral hippocampus (Figure 1(a,b)) compared with the control and M1145 groups (Newman-Keuls post-hoc test: $p < 0.05$) (Figure 3(a,b)). However, M1145 icv infused alone lacked effects on the numbers of BrdU-IR profiles (Figure 1b) compared with the control group (Figure 1(a–c)).

3.2. Identification of cellular types affected by increased survival after M1145 and Y1R agonist administration

Additionally, we studied the cellular types affected by the icv infusion of M1145 and the Y1R agonist. Quantification of BrdU labeled cells coexpressing either doublecortin (DCX)-expressing neuroblasts or marker of mature neurons (NeuN)-expressing cells was performed (Figure 1(e)). Results revealed that the number of BrdU+/NeuN+ cells increased significantly after the combined Y1R-GALR2 agonists treatment compared to the control group (t-test, $t = 3.153$, $df = 6$; $p < 0.05$)) (Figure 1(f)), indicating a preference for newly generated cells to differentiate toward a neuronal lineage (mature neurons). No significant change was noted in BrdU+/DCX+ cells (t-test, $t = 0.654$, $df = 6$).

3.3. Association of increased survival of mature neurons with enhanced brain-derived neurotrophic factor (BDNF) through coactivation of M1145 and Y1R agonists

To explore the mechanisms associated with the rise in neuronal survival, we conducted an examination of BDNF expression within the ventral hippocampal dentate gyrus (DG) following the icv administration of either M1145 or Y1R agonist. We detected BDNF-positive cells specifically localized in the granular region of the ventral hippocampus, along with sporadic cells in the polymorphic layer (P) of the ventral DG (Figure 2(a)). Through stereological quantification, we found a notable increase in the BDNF positive cells subsequent to the icv combined administration of M1145 and NPY1R agonist, when compared to control (one-way ANOVA, $F4,\ 15 = 12.39$, $p < 0.001$, Newman-Keuls post-hoc test: $p < 0.001$), M1145 (Newman-Keuls post-hoc test: $p < 0.001$) or NPY1R1 agonist alone (Newman-Keuls post-hoc test: $p < 0.05$) (Figure 2(a–d)). In alignment with the observations mentioned earlier, the presence of GALR2 antagonist M871 fully suppressed the increase prompted by the icv combined injection (Newman-Keuls post-hoc test: $p < 0.05$) (Figure 2b), highlighting GALR2's role in this interaction.

Furthermore, the icv administration of the Y1R agonist alone induced an increase in the number of BDNF positive cells in the granular zone of the ventral hippocampus (Figure 2(a,b)) compared with the control and M1145 groups (Newman-Keuls post-hoc test: $p < 0.05$) (Figure 2(a,b)). Conversely, the icv infusion of M1145 agonist alone showed no impact on the count of BDNF-positive cells in the ventral DG.

3.4. Enhancing effect of coadministration of GALR2 and the NPY1R agonists on survival and length of neurite processes

To detect overall neuronal viability modulating effects, the effects of the GALR2 agonist M1145 and/or the NPY1R agonist were monitored in an MTS cell proliferation assay after incubating cells with each substance in a time-course manner during 9 days. The results of these experiments are summarized in Figure 3(a). Upon incubation with M1145 and the NPY1R agonist, hippocampal neurons' viability was stable and resulted in an increased rate

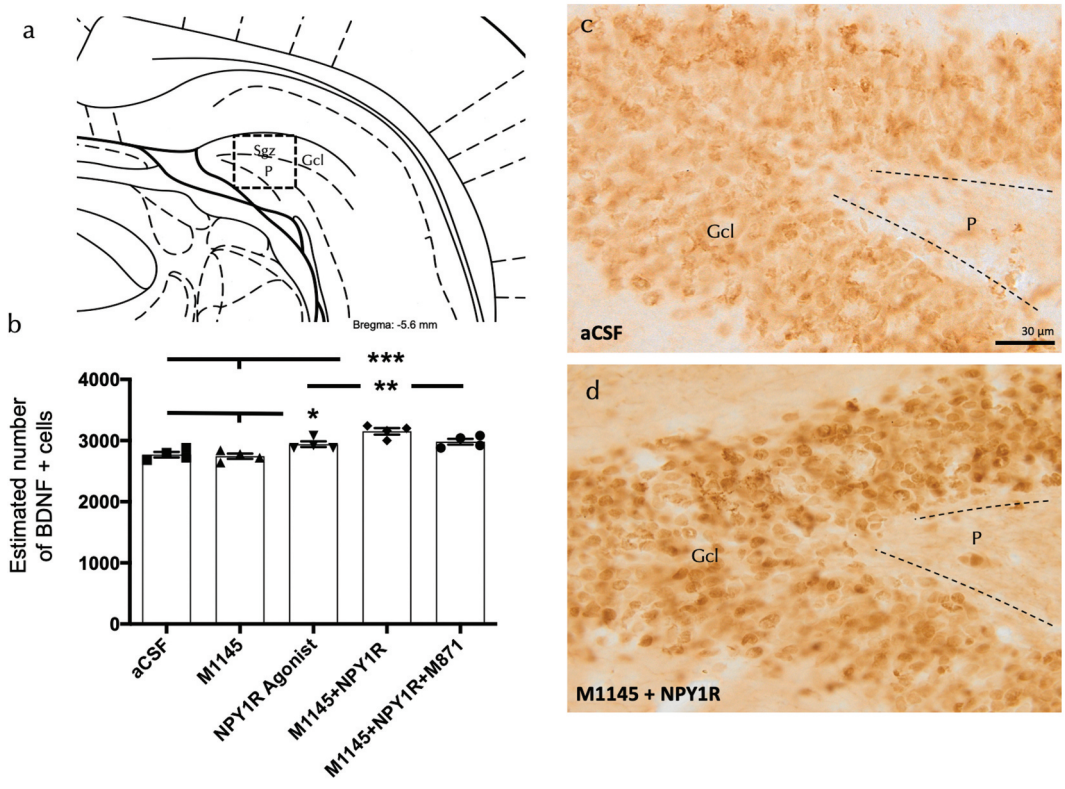

Figure 2. M1145 and the Y1R agonist effects on hippocampal brain-derived neurotrophic factor immunoreactive (BDNF-IR) cells of the ventral dentate gyrus (DG) hippocampal region.

Impact of co-administration of M1145 (a Galanin receptor 2 (GALR2) agonist) and Neuropeptide Y1 Receptor (NPY1R) agonist on the population of brain-derived neurotrophic factor immunoreactive (BDNF-IR) cells within the ventral dentate gyrus (DG) of the hippocampal region. Panel (a) illustrates that BDNF-IR cells are primarily localized in the granular cell layer (Gcl) of the dentate gyrus, with a few cells dispersed in the polymorphic layer (P) of the dentate gyrus in the ventral hippocampus, with the location corresponding to the Bregma coordinate -5.6 mm as delineated in the stereotaxic atlas by Paxinos and Watson [85]. Panel (b) presents a quantitative analysis demonstrating a significant increase in BDNF-IR cells within the ventral DG following the icv co-administration of M1145 and the NPY1R agonist. The presence of the GALR2 antagonist M871 was observed to mitigate this effect. Statistical significance is denoted by *$p < 0.05$ NPY1R agonist versus aCSF and M1145; **$p < 0.01$ M1145+NPY1R versus NPY1R agonist and M1145+NPY1R+M871; ***$p < 0.001$ M1145+NPY1R versus aCSF and M1145 as determined through one-way ANOVA followed by Newman – Keuls post-hoc test (with each group comprising $n = 4$). The vertical lines extending from the horizontal line above the bars in the graph illustrate comparisons between groups. All data are expressed as mean ± SEM. Panels (c) and (d) feature representative microphotographs that highlight the marked increase in BDNF-positive cells in the DG following the co-injection of M1145 and the Y1R agonist (d), in comparison to the control aCSF group (c). Dashed lines indicate the boundaries of the Gcl of the dentate gyrus. The abbreviations used are as follows: aCSF for the control group using artificial cerebrospinal fluid, M1145 for the Galanin receptor 2 agonist administered at 3 nmol, NPY1R agonist for the Neuropeptide Y1 Receptor agonist [Leu31, Pro34]NPY also at 3 nmol, M1145 + NPY1R for the combination treatment of M1145 and the Y1R agonist, and M1145 + NPY1R + M871 for the combined administration of M1145, the Y1R agonist, and the GALR2 antagonist M871 at 3 nmol.

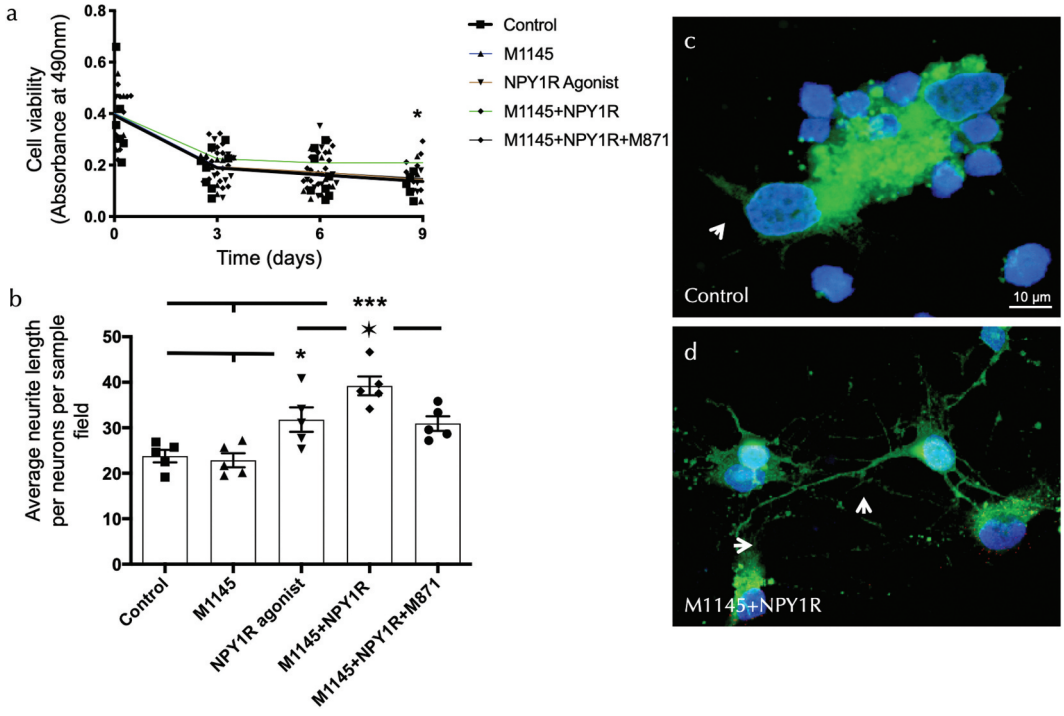

Figure 3. Evaluation of survival and neurite outgrowth on hippocampal neuronal cells.

Assessment of cell survival and neurite outgrowth in hippocampal neuronal cultures following treatment with galanin receptor 2 (GALR2) agonist (M1145) and Neuropeptide Y1 Receptor (NPY1R) agonist, either individually or in combination, and in the presence or absence of the GALR2 antagonist (M871). Panel (a) describes the use of the MTS assay (CellTiter 96A Aqueous One Solution Cell Proliferation Assay, Promega) to measure cell viability. Hippocampal neurons were treated with M1145 (100 nM) and the Y1R agonist (100 nM), alone or in combination, with or without M871 (1 µM). Each well, containing 20,000 cells, was assessed after 0, 3, 6, and 9 days of treatment in triplicate. Blanks (medium only plus CellTiter 96A Solution) were used to adjust the readings for each treatment group. Results showed a significant increase in neuronal survival in the ventral DG following M1145 and Y1R agonists' co-treatment, an effect inhibited by the GALR2 antagonist M871, with statistical significance indicated by *$p < 0.05$ at 9 days post co-stimulation M1145+NPY1R compared to all other groups ($n = 8$ replicates per group), as analyzed by Student's unpaired t-test. Panels (b) to (d) focus on the modulation of neurite length by GALR2 and Y1R agonists. Neurite length per cell was quantified after immunofluorescent labeling with a pan-neuronal marker (ABN2300) and nuclear staining (DAPI). Quantitative results, detailed in Figure 3(b), are presented as mean ± SEM, highlighting significant differences between the combined treatment group and other groups (*$p < 0.05$ NPY1R agonist vs. control and M1145; ✶$p < 0.05$ M1145+NPY1R vs. NPY1R agonist and M1145 +NPY1R+M871; ***$p < 0.001$ M1145+NPY1R vs. control and M1145), determined through one-way ANOVA and Newman – Keuls post-hoc test ($n = 5$ per group). Representative microphotographs (c and d) exhibit a notable increase in neurite length in hippocampal cells following treatment with M1145 and Y1R agonist (d) compared to the control group (c). Neurons are visualized in green through confocal laser microscopy, with white arrows indicating neurite extensions, and nuclei counterstained in blue with DAPI. Abbreviations: Control = Culture medium, M1145 = galanin 2 receptor agonist (100 nM), NPY1R agonist = NPY1R receptor agonist [Leu31-Pro34]NPY (100 nM), M1145 + NPY1R = co-administration of M1145 and NPY1R, M1145 + NPY1R + M871 = co-incubation with M1145, Y1R, and GALR2 antagonist (1 µM).

of survival at 9 days, as compared with the control ($t = 2.535$, df = 14; $p < 0.05$), M1145 ($t = 2.765$, df = 14; $p < 0.05$), and NPY1R agonist ($t = 2.649$, df = 14; $p < 0.05$) groups (Figure 3(a)). Moreover, the specific GALR2 antagonist M871 abolished this effect ($t = 2.755$, df = 14; $p < 0.05$) (Figure 3(a)), demonstrating that this effect was mediated through the coactivation of GALR2 and NPYY1R. These results indicate that the co-incubation of the GALR2 agonist and the NPY1R agonist in solution exerts a significant effect on the survival of neurons. The incubation with the NPY1R agonist alone revealed a non-significant tend to increased neuronal viability. Moreover, incubation with M1145 alone revealed a minor influence on neuronal viability compared to the control.

Due to the changes observed in the survival of hippocampal cells upon treatments, we decided to study the morphology and structural plasticity changes. The data show a significant synergistic increase of mean neurite length upon coactivation of M145 and the Y1R agonist compared to the NPY1R agonist group alone (one-way ANOVA, F4, 20 = 12.18, $p < 0.001$, Newman-Keuls post-hoc test: $p < 0.05$), control (Newman-Keuls post-hoc test: $p < 0.001$) and M1145 alone (Newman-Keuls post-hoc test: $p < 0.001$) (Figure 3(b,c,d)). Furthermore, the presence of GALR2 antagonist M871 entirely blocks the synergic effects (Newman-Keuls post-hoc test: $p < 0.05$) (Figure 3(b)). The Y1R agonist incubated alone increased the neurite length compared to control and M1145 alone (Newman-Keuls post-hoc test: $p < 0.05$) (Figure 3(b)).

3.5. Enhanced antidepressant-like response induced by GALR2 and Y1R agonists in the forced swim test

In the Forced swim Test (FST), we assessed the functional outcomes following icv delivery of GALR2 and NPY1R agonists. Rats underwent a 10-minute FST session 3 weeks icv post-administration. The analysis of immobility and swimming behaviors revealed significant changes. Specifically, coadministration of M1145 and Y1R agonists resulted in a marked decrease in immobility time (one-way ANOVA, F4,25 = 10.12, $p < 0.001$; Figure 4(a)) compared to the NPY1R agonist group alone (Newman-Keuls post-hoc test: $p < 0.05$), control (Newman-Keuls post-hoc test: $p < 0.001$) and M1145 alone (Newman-Keuls post-hoc test: $p < 0.001$). Enhanced swimming activity of M1145 and Y1R agonists icv combination (one-way ANOVA, F4,25 = 11.86, $p < 0.001$; Figure 4(b)) compared to the NPY1R agonist group alone (Newman-Keuls post-hoc test: $p < 0.05$), control (Newman-Keuls post-hoc test: $p < 0.001$) and M1145 alone (Newman-Keuls post-hoc test: $p < 0.001$) was also

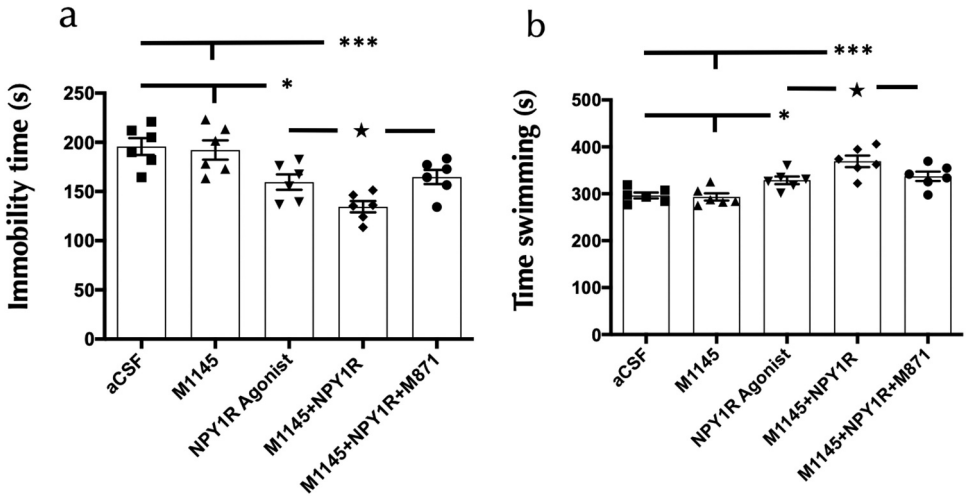

Figure 4. Behavioral responses to combined GALR2 and NPY Y1 receptor agonists in the forced swim test (FST).

Behavioral outcomes resulting from the intracerebroventricular (icv) administration of the galanin receptor 2 (GALR2) agonist (M1145) and the neuropeptide Y1 receptor (NPY1R) agonist, applied both separately and in combination, during the Forced swim Test (FST). The data compellingly demonstrate the antidepressant-like effects manifesting three weeks following the joint administration of M1145 and the NPY1R agonist, effects that were notably diminished upon the simultaneous introduction of the GALR2 antagonist, M871. The graph provides a quantitative representation of the behavioral metrics assessed, including the total duration of immobility (a) and active swimming (b), across the experimental groups. Each data point represents the mean ± SEM for groups consisting of six animals. Statistical significance is highlighted as follows: In panel a, *$p < 0.05$ signifies a significant difference of NPY1R agonist from the Control and M1145 groups; ★P < 0.05 indicates a significant difference of M1145+NPY1R from the NPY1R agonist and M1145+Y1R+M871 groups; ***$p < 0.001$ marks a significant difference of M1145+NPY1R from the Control and M1145 groups. Similarly, in panel b, *$p < 0.05$ denotes significance of NPY1R agonist versus the Control and M1145 groups; ★P < 0.05 indicates significance of M1145+NPY1R versus the NPY1R agonist and M1145+Y1R+M871 groups; ***$p < 0.001$ signifies a significant difference of M1145+NPY1R from the Control and M1145 groups. These significances were determined using one-way ANOVA followed by the Newman-Keuls post-hoc test ($n = 6$), with horizontal and vertical lines above the bars facilitating inter-group comparisons. Abbreviations used are: aCSF = Control (artificial cerebrospinal fluid), M1145 = Galanin receptor 2 agonist (3 nmol), NPY1R agonist = NPY1R receptor agonist [Leu31, Pro34]NPY (3 nmol), M1145 + NPY1R = co-administration of M1145 and Y1R agonist. M1145 + NPY1R + M871 = co-administration of M1145, Y1R, and GALR2 antagonist M871 (3 nmol).

observed. The addition of GALR2 antagonist M871 counteracted these effects, as indicated by the post-hoc tests ($p < 0.05$ for both immobility and swimming). Figure 4(a) illustrates the significant reduction in immobility time in the FST, while Figure 4(b) shows the corresponding increase in swimming behavior following treatment.

The icv infusion of the NPY1R agonist alone decreased the time of immobility compared to the control (Newman-Keuls post-hoc test: $p < 0,05$; Figure 4(a)) and the M1145 (Newman-Keuls post-hoc test: $p < 0,05$; Figure 4(a)) groups; while induced an increase in the swimming behavior compared to the control (Newman-Keuls post-hoc test: $p < 0,05$; Figure 4(b)) and the M1145 (Newman-Keuls post-hoc test: $p < 0,05$; Figure 4(b)) groups. However, the administration of the GalR2 agonist M1145 alone lacked effects on the FST (Figure 4(a,b)) compared with the control group. No differences were observed between groups in climbing time on the FST (Supplementary Table S1). Moreover, no differences were observed between groups in locomotor activity on the open field test (Supplementary Table S2).

4. Discussion

Our research sheds light on how GALR2 and NPY1R agonists work together to boost cell survival, growth, and BDNF levels in the brain, offering new hope for treating depression. This research not only corroborates the pivotal role of neurogenesis in mood regulation but also highlights the therapeutic potential of targeting specific neuropeptide receptors to enhance neurogenic processes and alleviate depressive symptoms. The elevation of BDNF, a key factor in neuronal survival and differentiation, highlights a common mechanistic pathway that may underlie the antidepressant effects of diverse treatments, from physical exercise to pharmacological interventions. By comparing these outcomes with existing literature, our findings suggest that the co-activation of GALR2 and NPY1R offers a novel approach to modulate the neurobiological underpinnings of depression, potentially overcoming limitations of current antidepressant therapies. This critical analysis not only situates our work within the broader scientific dialogue but also proposes a direction for future research to explore the clinical applicability of these findings.

Our research contributes valuable insights into the beneficial effects of combined intracerebroventricular administration of GALR2 and NPY1R agonists on neuronal survival and maturation within the dentate gyrus of the ventral hippocampus. Having outlined the specific impacts of GALR2 and NPY1R agonist co-activation on neuronal survival and differentiation, it's crucial to contextualize these findings within the larger framework of neurogenesis and its role in depression. This leap from micro to macro-level understanding allows us to appreciate the potential of targeting neurogenesis as a therapeutic strategy for mood disorders. In our study, the intracerebroventricular (ICV) co-administration of M1145 and the Y1R agonist led to a significant increase in the number of BrdU-immunoreactive (BrdU-IR) profiles within the subgranular zone of the dentate gyrus. The magnitude of this change, and the observed reversal of this effect by the GALR2 antagonist M871, emphasize the specific contribution of GALR2 in synergy with NPY1R to promote neuronal survival. This increase aligns with findings that, by the third week post-administration, a significant number of neurons not only survive but also actively contribute to hippocampal functions in rodent models [15]. Moreover, our findings demonstrated a significant increase in the number of cells co-expressing BrdU and the neuronal nuclear protein NeuN, a marker of mature neurons, following combined treatment. This signifies not just an

increase in neuronal survival but also a progression toward neuronal maturation, indicating that these neuropeptide agonists foster an environment conducive to neuronal differentiation. This observation of increased BrdU+/NeuN+ cells after combined agonist treatment compared to the control group highlights the substantial nature of neurogenic enhancement and neuronal maturation prompted by the treatment. This trend is consistent with other studies that have highlighted NeuN's role as a marker for mature neurons, visible three weeks following such interventions [86]. Yet, our findings also reflect lower counts of cells co-expressing BrdU and doublecortin (DCX), a marker for neuronal precursors, at the three-week mark, corroborating earlier observations [15,87]. The potential mechanisms underlying these remarkable changes may involve the upregulation of Brain-Derived Neurotrophic Factor (BDNF), a key player in supporting neurogenesis and neuronal survival.

Building upon the understanding of neurogenesis in depression, our findings invite comparisons with other compounds known for their neurogenic and antidepressant properties. Such comparisons not only highlight the unique contributions of our study but also frame our results within the broader pursuit of effective depression treatments. Intriguingly, recent research has highlighted the potential of enhancing neurogenesis within the ventral hippocampal DG to reinforce resilience against depressive states [88]. Compounds like P7C3 have been shown to augment cell proliferation in the hippocampal DG, correlating with antidepressant effects across both rodent and primate studies. The neuroprotective effects of P7C3 are believed to stem from its ability to safeguard mitochondrial membrane integrity, a critical factor in maintaining cellular energy balance and preventing apoptosis. While the precise molecular target of P7C3 remains to be fully elucidated, research in rodent models and cellular systems has demonstrated that P7C3 effectively increases neuronal NAD flux under conditions that would otherwise lead to cell death [89,90]. While the exploration of other compounds provides valuable context, the enhanced neuronal survival and BDNF elevation observed with the long-term co-activation of GALR2 and NPY1R highlight the potential of these receptors as targets for innovative antidepressant strategies. At the cellular level, the observed increases in hippocampal neuronal survival and differentiation may be mediated by elevated levels of Brain-Derived Neurotrophic Factor (BDNF) in the ventral hippocampal DG post GALR2 and NPY1R agonists treatment. BDNF plays a crucial role in enhancing neurogenesis, impacting both cell proliferation and survival, and is a pivotal mediator in the neuroprotective effects of antidepressants [91–93]. Physical activity, for instance, has been shown to mitigate depressive symptoms by augmenting hippocampal neurogenesis alongside BDNF levels [94]. Conversely, reductions in hippocampal BDNF levels have been reported in postmortem analyzes of MDD sufferers and suicide victims [95,96], while BDNF injections into the hippocampus have demonstrated a capacity to alleviate depression-like behaviors in rodent models [97]. Our findings are in line with prior evidence suggesting NPY's BDNF-mediated neuroprotective effect in neurodegeneration models [46,98,99]. Moreover, physical exercise has been shown to enhance BDNF signaling and neuronal proliferation in the ventral hippocampus, correlating with antidepressant effects [94].

The rationale behind conducting experiments on hippocampal cell viability and neurite length, and the subsequent findings, form a pivotal aspect of our study, emphasizing the therapeutic potential of GALR2 and NPY1R agonists. These investigations were designed to explore not only the neurogenic effects of these agonists but also their impact on the structural and functional maturation of neurons, a critical dimension in understanding antidepressant mechanisms. Enhanced cell viability in B27-deprived conditions indicates a robust neuroprotective effect, suggesting that the treatments could counteract stress-induced cellular vulnerability, a hallmark of depressive states. Similarly, the observed increase in neurite length aligns with the enhancement of neuronal connectivity and network integration, essential for the restoration of normal hippocampal function implicated in mood regulation. These cellular outcomes, potentially mediated by BDNF highlight a mechanistic pathway through which the combined agonist treatment exerts its effects [100,101]. By examining these parameters, our study provides a comprehensive insight into how GALR2 and NPY1R agonists promote neuronal health and connectivity, offering a foundation for their antidepressant-like efficacy observed in behavioral assays.

Functionally, the antidepressant-like effects of the GALR2 and NPY1R agonist combination were validated using the forced swim test (FST), according with findings that implicate the ventral hippocampus in the antidepressant efficacy of NPY in models of posttraumatic stress disorder [102]. This observation highlights the therapeutic potential of modulating specific neuropeptide receptors, such as GALR2 and NPY1R, in eliciting antidepressant responses. Similarly, treatments like NPY and NPY1R agonists have shown promise in inducing antidepressant effects across various species, reinforcing the value of targeting these pathways [70,103,104]. In our study, the differential increase in swimming behavior observed in the rat Forced Swim Test suggests that the scoring of swimming time can provide valuable insights into the underlying mechanisms mediating antidepressant-like effects. Specifically, akin to the action of selective 5-HT reuptake inhibitors, the decrease in immobility and concurrent increase in swimming behavior observed could indicate a serotonergic mechanism at play, underlining the complexity of neurochemical interactions involved in modulating antidepressant-like effects [105]. It is pertinent to note the absence of locomotor alterations elicited by the treatments. This observation is critical as it underscores that the observed antidepressant-like effects are not confounded by changes in general activity levels, thus providing a clearer interpretation of the specific therapeutic potential of the treatments without the influence of altered locomotion.

While our findings illuminate the antidepressant-like effects of the NPY1R and GALR2 agonist combination, it is essential to weave this narrative into a broader context that acknowledges species-specific responses to these treatments. Research employing intranasal GALR2 agonist treatments in mice has unveiled critical insights into the nature of antidepressant efficacy across different species [57,106]. Furthermore, the exploration of stabilized GALR2 agonists like M39b opens new horizons for therapeutic interventions, demonstrating the evolving landscape of neuropeptide-based treatments [107]. The promising therapeutic

potential of these agonists not only aligns with our study's outcomes but also propels the discussion toward the future of depression treatment. It underscores the importance of continuing to explore the intricate dynamics between neuropeptide receptors and their agonists, particularly in the context of developing more effective and targeted antidepressant therapies. By situating our study within these broader discussions, we emphasize the interconnectedness of our findings with ongoing research into the mechanisms of action and therapeutic applications of GALR2 agonists. This approach aims to highlight the significance of our results while acknowledging the complexity and diversity of research directions that stem from understanding the roles of GALR2 and NPY1R in depression treatment. Future research must delve into critical unanswered questions, such as the precise mechanisms of receptor interaction, optimal dosing strategies for maximal efficacy with minimal side effects, and the potential for synergistic effects with other therapeutic agents. Addressing these inquiries will be pivotal in translating our findings into viable clinical applications.

It is crucial to acknowledge certain limitations that might affect the interpretation and generalizability of our findings. First, the choice of animal model, while instrumental in elucidating the neurobiological underpinnings of antidepressant-like effects and the potential therapeutic effects of NPY1R and GALR2 agonists, may not fully capture the complexity of Major Depressive Disorder as it manifests in humans. Rodent models, despite their utility, inherently possess differences in brain structure and function that could influence the translation of these findings to clinical settings. Second, while our experimental design focused on the intracerebroventricular administration of the agonists, representing a highly controlled method of drug delivery, we acknowledge it might not directly correspond to methods available for human treatment, raising questions about the feasibility of translating these specific administration routes and dosages to clinical practice. However, exploring the potential of intranasal administration of these peptides presents a promising avenue for enhancing their translational value to clinical settings, offering a more direct and noninvasive route that could facilitate the therapeutic application of our findings. Additionally, while we have made efforts to minimize variability and ensure rigorous control within our experimental setup, the relatively small sample size and the exclusion of female subjects limit the breadth of our conclusions. The inclusion of a broader demographic, considering sex differences, could provide a more comprehensive understanding of the treatment effects across populations. Lastly, while our study highlights the promising therapeutic potential of co-activating GALR2 and NPY1R, the mechanisms underlying these effects warrant further investigation to fully understand their implications for depression therapy. These limitations highlight the need for continued research, including larger, more diverse studies and exploration into the mechanisms of action, to build upon our findings and advance toward clinically relevant applications. In our research, the utilization of naive animal models, which were not subjected to any specific depression-inducing procedures, was instrumental in shedding light on the potential pathophysiological mechanisms underlying mood disorders and the action mechanisms of antidepressant agents. This methodology holds particular significance in the investigation of subclinical symptoms and the exploration of the preventative capacities of therapeutic interventions. Previous studies leveraging naive models have been pivotal in enhancing our comprehension of the neurobiological pathways and behavioral dynamics related to mood regulation [44,108,109]. These contributions highlight the value of employing naive models during the preliminary phases of research into new therapeutic targets and mechanisms. This foundational work sets the stage for further, more detailed investigations in models that closely mimic the complex pathology of depression, such as exposition to chronic unpredictable stress, corticosterone or LPS injection.

5. Conclusions

This study provides compelling evidence that the synergistic administration of Galanin Receptor 2 (GALR2) and Neuropeptide Y1 Receptor (NPYY1R) agonists facilitates hippocampal neurogenesis and induces antidepressant-like behaviors in a rodent model. The findings indicate a significant increase in neuronal survival and differentiation, alongside an upregulation of Brain-Derived Neurotrophic Factor (BDNF), suggesting a potential mechanistic pathway for the observed antidepressant effects. Importantly, these therapeutic effects were achieved without the report of adverse events, highlighting the safety profile of these treatments within the study's scope.

The clinical implications of these results are substantial, offering a promising avenue for the development of new therapeutic strategies for Major Depressive Disorder (MDD). By targeting specific neural pathways involved in neurogenesis and mood regulation, GALR2 and NPYY1R agonists could represent a novel class of antidepressants with potentially fewer side effects compared to traditional treatments.

However, it is important to acknowledge that while the study's outcomes are encouraging, they are derived from a preclinical model. The generalizability of these results to the broader patient population with MDD remains to be established through clinical trials. Further research is warranted to validate the efficacy and safety of GALR2 and NPYY1R agonists in humans, as well as to explore the long-term implications of their use in the treatment of depressive disorders.

Funding

This manuscript was funded by the UMA18-FEDERJA-100 and ProyExcel_00613, Junta de Andalucía, Spain, to MN. Additional funding support came from Cátedra Imbrain: Neurociencia Integrada y Bionestar to MN. This work also received support from Stiftelsen Olle Engkvist Byggmästare in 2018 and 2021, as well as from the Swedish Medical Research Council [Grant No. 62X-00715-50-3] awarded to KF and DB-E. Additionally, funding was provided by Hjärnfonden [Grants F02018-0286 and F02019-0296], Karolinska Institutet Forskningsstiftelser 2022, EMERGIA 2020-39318 [Plan Andaluz de Investigación, Desarrollo e Innovación 2020], and CONSOLIDACION INVESTIGADORA [CNS2022-136008, Programa Estatal para Desarrollar, Atraer y Retener Talento, del Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023] awarded to DB-E. DB-E is affiliated with the Academia de Biólogos Cubanos and the Observatorio Cubano de Neurociencias (Yaguajay, Cuba).

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Author contributions

D Borroto-Escuela and M Narváez played a pivotal role in the conception and design of the study. They were instrumental in formulating the research questions and setting up the experimental framework. P Serrano-Castro, JA Sánchez-Pérez, K Fuxe and MA Barbancho-Fernández contributed extensively to the analysis and interpretation of the data, applying their expertise to decipher the complex results and understand their implications. M Narváez was involved in drafting the manuscript, providing critical insights that enhanced the intellectual content of the paper. All authors participated in revising the manuscript critically, offering valuable feedback and suggestions that significantly improved the quality of the final submission. They also gave their final approval of the version to be published, ensuring that it accurately reflects their findings and conclusions.

Availability of data and materials

The data that support the findings of this study are openly available in the Institutional repository of the University of Malaga (RIUMA) and from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All experimental protocols were approved by the Local Animal Ethics, Care, and Use Committee for the University of Málaga, Spain (CEUMA 45–2022-A), and conducted in accordance with the EU Directive 2010/63/EU and Spanish Directive (Real Decretory 53/2013).

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