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G-protein-Coupled Receptors or Cytokine Receptors: Which Target Is More Promising For Future Neuroregenerative Drugs?

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Abstract: Despite advances in neuroscience and neuropharmacology, there are still no effective treatments for neurodegenerative diseases. This also applies to pathologies such as Alzheimer's disease (AD) and toxic encephalopathies, which, it would seem, do not have obligatory hereditary causes of development. The creation of pharmacological approaches to stimulating neuroregeneration of nervous tissue affected by pathological processes looks promising. Theoretically, this can be achieved by targeting the receptors of regenerative-competent cells of nervous tissue responsible for the implementation of their growth potential. The aim of the work was a comparative study of the effect of G Protein-coupled Receptor (GPCR) Kinase 5 (GRK5) and JAKs blockers on the functioning of nervous tissue progenitors and the impairment of the psychoneurological status of experimental animals under conditions of modeling alcoholic encephalopathy (AE) and AD. The blockade of GPCR and cytokine receptor signaling almost equally stimulated the functions of resident progenitors of the brain in the subventricular zone of the cerebral hemispheres under conditions of modeling neurodegenerative conditions. In both cases, significant stimulation of the growth potential of neural stem cells and neuronal committed precursors was observed. At the same time, only the GRK5 inhibitor caused correction of exploratory behavior disorders and conditioned reflex activity in animals after modeling AE and AD. The findings indicate the potential of using GPCR as pharmacological targets for potential agents with neuroregenerative activity.

Keywords: neurodegenerative diseases; GPCR; cytokine receptors; GRK5; JAKs; neural stem cells; committed neuronal precursors; signal transduction.

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1. Introduction

Neurodegenerative diseases have become a real scourge of modern society in recent decades. At the same time, the age of manifestation of many of them has significantly decreased in recent years [1,2]. Despite the achievements in neuroscience and neuropharmacology, there are still no highly effective means that allow not only to cure, but also to significantly slow down the progression of such diseases. Moreover, this also applies

to diseases that do not have obvious obligatory hereditary causes, such as Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, and others, including even toxic encephalopathies [1-6].

A characteristic feature of neurodegenerative pathological conditions is the development of disorders of the central nervous system against the background of disturbances in the functioning of the cellular renewal system of nervous tissue [7,8]. A deep reserve for brain regeneration is the population of multipotent neural stem cells (NSC) that persists (in some areas) throughout almost the entire life [7-10]. In addition, there are pools of cells that are determined in their development (committed precursors: neurons, oligodendrocytes, and astrocytes), which can also play an important role in neurogenesis and neuroplasticity [11-13]. However, in neurodegenerative conditions, these cell renewal systems are unable to compensate for the effects of pathogenic factors. Often, there is a decrease and/or discoordination of the functional activity of different types of these regenerative-competent cells [12-14].

Today, several different strategies of pharmacological intervention are being developed to stimulate neurogenesis in degenerative diseases. Depending on the molecular target and origin of the agents, the following can be distinguished: 1) the use of recombinant neurotrophic and other growth factors [15,16]; 2) the development of synthesized selective modifiers of the activity of individual intracellular signaling molecules [12,17]; 3) creation of targeted low-molecular synthetic substances – ligands of regenerative-competent cell receptors [7,18,19]. The first of the designated directions appears to be the least promising due to the understandable (primarily, the impossibility of penetrating the blood-brain barrier) pharmacokinetic characteristics of such protein-based agents [15,17]. The second concept is undoubtedly the most promising in the long term due to the possibility of maximally selective influence on different compartments of the cellular renewal system of nervous tissue (caused by the specificity of phenotypes/patterns of intracellular signaling in different types of progenitors [7,8,12]). However, its implementation requires a more detailed understanding of the features of intracellular signaling than the ideas currently available in this area [7,17]. The third approach is sufficiently theoretically substantiated and does not have the shortcomings characteristic of the first of the designated directions. It has every reason to give a result of the proper quality already at the present time and is quite suitable for the creation of effective neuroregenerative agents. Nevertheless, there are a number of points that should be taken into account when choosing certain receptors as targets for such pharmacological action [17-19].

It is known that most growth factors initiate cell proliferation and differentiation through interaction with receptors belonging to the cytokine receptor superfamily [20,21]. Such cytokine signaling is mediated by the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. These receptors do not have intrinsic kinase activity; their subunits are non-covalently bound to JAKs. When a ligand binds to a receptor, JAKs are activated, accompanied by the addition of phosphates to the receptor tyrosine, which in turn is accompanied by phosphorylation of STAT proteins [22,23]. Dimers of transcription factors of the STAT family are formed, translocating into the nucleus and triggering the expression of genes responsible, among other things, for the realization of the growth potential of different progenitors [22-24].

At the same time, the realization of the growth potential of progenitor cells can also be carried out through the activation of G-Protein-Coupled Receptors (GPCR) [25-27]. This group of receptors is a very large family. Signal transmission in this case is carried out

through G-proteins. The heterotrimeric G protein family consists of different subunits with multiple isoforms (α-, β-, γ-subunits) that can combine in different combinations to form a dizzying number of potential G protein heterotrimers [28]. Signal transduction is mediated by G Protein-coupled Receptor Kinases (GRKs). GRKs are serine/threonine kinases that phosphorylate ligand-bound GPCRs. There are 7 types of GRKs (GRK1-GRK7). GRK1 and GRK7 are primarily located in the retina, and GRK4 is predominantly found in the testes. GRK2, GRK3, and GRK5 are expressed ubiquitously [25-28] (with GRK5 being expressed in significant quantities in nervous tissue). Activation of GRK is accompanied by phosphorylation of downstream signaling molecules such as adenylate cyclase, phosphoinositide 3-kinase (PI3K), phospholipase Cβ (PLCβ), Ras-ERK, and some others [26,27].

The analysis of data on the control of the functions of neural tissue progenitors in vitro and neurogenesis in vivo through targeted action on some individual intracellular molecules related to GPCR and cytokine receptors–pathways, revealed certain patterns. In both cases, there is data on the possibility of stimulating pro-regenerative properties, for example, of NSC [7,8,12]. However, when administered in vivo to models of neurodegeneration, only various agents affecting GPCR-signaling showed therapeutic/neuroplasticity effects (for example, when blocking NF-kB, JNK, and AC) [7,29-33]. While the use of STAT blockers (i.e., proteins that mediate signals from cytokine receptors) in the conditions of modeling alcohol-induced neurodegeneration (alcoholic encephalopathy (AE)) was not able to effectively stimulate neurogenesis [31].

The aim of the study was a comparative study of the effect of GRK5 and JAKs blockers on the functioning of nervous tissue progenitors and the impairment of the psychoneurological status of experimental animals under conditions of modeling AE and AD.

2. Materials and Methods

2.1. Chemicals.

Ethyl alcohol (OJSC "Kemerovo Pharmaceutical Factory", Russia); Scopolamine hydrobromide (SH) (Sigma-Aldrich, USA); GRK5 Inhibitor HY-136561 (GRK5-IN-2) (MedChemExpress LLC, USA); Pan JAK Inhibitor «Ruxolitinib» (InvivoGen, USA); MACS Neuro Medium (MiltenyiBiotec B.V. & Co. KG, Germany); anti-PSA-NCAM MicroBeads (MiltenyiBiotec B.V. & Co. KG, Germany); hydroxycarbamide hydurea (Calbiochem, USA); dimethyl sulfoxide (DMSO) (Calbiochem, USA); Cell Culture Plate (96 wells) (Corning, USA).

2.2. Animals and experimental design.

The experiments were carried out in accordance with the principles of the humane treatment of animals (EU Directive 2010/63/EU). Permission was previously obtained from the local ethics committee of the Institute (protocol IACUC-2023-04/18). The studies were carried out on 231 C57BL/6 mice aged 2-2.5 months.

The animals were randomly divided into 7 groups: 1) intact mice (intact control), 2) mice with AE 3) mice with AD; 4) mice receiving the GRK5 inhibitor after modeling AE; 5) mice receiving the Pan JAK inhibitor after modeling AE; 6) mice receiving the GRK5 inhibitor after modeling AD; 7) mice receiving the JAKs inhibitor after modeling AD (n=33 in each group).

In all groups, the preservation of cognitive functions was studied using open field tests (on days 3, 7, 14, and 21) and the reproduction of the conditioned passive avoidance reflex (CPAR) of an electric pain stimulus (on days 7, 14, and 21) (n=15 each in the control and experimental groups) [7,33]. The content and functioning of nervous tissue progenitors were also determined (on days 3, 7, and 14) (n=18 each in the control and experimental groups) [12,14].

2.3. Models of neurodegenerative diseases.

To model AE, mice were given 0.2 ml of 30% ethyl alcohol (3 g/kg/day) daily through an intragastric tube for 16 weeks. Instead of drinking water, the animals had a 5% ethanol solution in their cages [30,34].

To simulate Alzheimer's disease, mice were intraperitoneally injected with SH daily for 28 days at a dose of 5 mg/kg [8,35]. Control group animals (intact mice) received distilled water in an equivalent amount in a similar manner.

The GRK5 and JAKs inhibitors were started to be administered 7 days after the end of the modeling of pathological conditions (the last administration of ethanol or SH). The GRK5 inhibitor was administered subcutaneously once daily for 5 days at a dose of 10 mg/kg [36-38]. The JAK inhibitor was also administered subcutaneously once daily for 5 days at a dose of 30 mg/kg [39,40]. Doses are selected taking into account their half-maximal inhibitory concentrations (IC_{50}). Control mice received the corresponding solvents in similar modes and in equivalent volumes (0.2 ml) of 0.2% DMSO. The observation periods in the description of the experiments were counted from the day of the start of the administration of inhibitors, which was considered day “0”.

2.4. Psychoneurological status.

The influence of pharmacological agents on cognitive functions was assessed using functional methods (n=15 in the control (intact control and control - animals with encephalopathy) and experimental groups). When using the open field test, the indicators of locomotor activity and various types of exploratory behavior were recorded (the parameters are indicated in the corresponding tables). In this case, the indicators of the first and 2–3 minutes were taken into account separately, which made it possible to differentiate the features of predominantly psychoemotional reactions and/or cognitive functions [7,33].

The conditioned reflex activity of the central nervous system (CNS) was assessed based on the preservation of the mnemonic function using the method of determining the reproducibility of the conditioned passive avoidance reflex (CPAR) [7,33].

2.5. Cell assays.

The functioning of multipotent NSCs and neuronally committed precursors (NCPs) in the subventricular zone of the cerebral hemispheres (SVZ) was studied. The number of NSC and NCP in the cellular material was determined by the level of colony formation in cultures of unfractionated and CD56+ cells, respectively. CD56+ cells were isolated using positive immunomagnetic selection using PSA-NCAM (MiniMACS Cell Separator (Miltenyi Biotec, Germany)). After this, the cells were incubated in MACS Neuro Medium (10^5/ml) under standard conditions (37°C, 5% CO_2, and 100% air humidity) for 5 days. The content of NSC and NCP in the studied cellular material was assessed by its colony-forming capacity (CFU,

neurospheres of more than 100 cells). The mitotic activity of CFU was also determined. For this purpose, we used hydroxyurea (at a concentration of 1 μM in the medium), which blocks the S-phase of the cell cycle. In addition, the ratio of the number of cluster-forming units (CFU, small neurospheres (30-80 cells)) to CFU was determined by the NSCs and NPCs specialization index (differentiation/maturation intensity) [12,14,29].

2.6. Statistical analysis.

The statistical processing of data was carried out using one-way ANOVA analysis of variance (using Dunnett's, Wilcoxon's, and Mann-Whitney tests). The analysis package STATISTICA 13.0 was used. In the tables, the results are indicated in the form of arithmetic means and standard error of the mean (M±SEM); in the figures, the arithmetic mean and the significance of the difference in indicators are indicated at $p < 0.05$.

3. Results and Discussion

3.1. Modeling of neurodegenerative conditions.

3.1.1. Cognitive and behavioral disorders.

3.1.1.1. AE model.

Modeling of AE was accompanied by the appearance of pronounced signs of disorders of the psychoneurological status in experimental animals. An increase in their locomotor activity was observed mainly due to horizontal movements in the "open field" setup. The number of other types of exploratory behavior, on the contrary, decreased (Table 1). This simplification of the nature of locomotor activity was accompanied by a natural increase in its asymmetry coefficient. It is important to note that the indicated shifts in locomotor activity were noted both in the first and in the second testing period. Since in the first case the revealed phenomena spoke to a greater extent about the high anxiety of the animals, and in the second, mainly about the disorder of their cognitive (research) activity [8,29,41].

Table 1. Locomotor activity in the “open field” of intact C57BL/6 mice (1); in C57BL/6 mice with AE (2); with the GRK5 inhibitor administration to mice with AE (3), with the Pan JAK inhibitor administration to mice with AE (3), arb. units (M±SEM).

Groups	Horizontal locomotor activity	Vertical locomotor activity	Hole-board exploration	Self-grooming	Defecation	Total locomotor activity	Asymmetry coefficient
Day 3							
Frist period							
1	14.91 ± 1.05	3.01 ± 0.39	4.55 ± 0.44	0.67 ± 0.23	0.34 ± 0.14	21.65 ± 1.67	0.47 ± 0.02
2	19.55 ± 1.05*	3.93 ± 0.66	3.32 ± 0.83	1.62 ± 0.21	1.33 ± 0.15*	29.18 ± 2.14 *	0.65 ± 0.03*
3	15.62 ± 1.45	2.50 ± 0.77	2.44 ± 0.22*	1.45 ± 0.26	1.12 ± 0.18*	27.93 ± 2.87*	0.68± 0.02*
4	17.85 ± 1.12*	2.89 ± 0.91	3.56 ± 0.75	1.42 ± 0.34	1.19 ± 0.17*	28.23 ± 3.74*	0.69 ± 0.04*
Second period							
1	26.15 ± 1.79	5.27 ± 0.67	5.58 ± 1.14	1.15 ± 0.21	0.64 ± 0.32	41.12 ± 3.67	0.69 ± 0.02
2	37.16 ± 2.68*	1.84 ± 0.40*	2.57 ± 0.46*	0.13 ± 0.08*	0.17 ± 0.12	42.47 ± 3.89	0.77 ± 0.04*
3	31.43 ± 5.32	2.34 ± 1.31#	3.76 ± 1.14	1.09 ± 0.41	0.43 ± 0.14	39.23 ± 5.29	0.78 ± 0.04*
4	34.26 ± 3.28*	1.56 ± 0.98*	3.01 ± 0.37*	1.23 ± 0.27	0.32 ± 0.17	41.26 ± 4.78	0.76 ± 0.03*
Day 7							
Frist period							
1	14.67 ± 1.20	2.80 ± 0.40	1.80 ± 0.50	1.27 ± 0.23	0.60 ± 0.27	21.13 ± 1.36	0.63 ± 0.02
2	19.67 ± 1.17*	3.33 ± 0.60	1.00 ± 0.32	0.42 ± 0.04*	0.33 ± 0.13	26.33 ± 1.12*	0.78 ± 0.03*
3	15.61 ± 2.34	2.04 ± 0.33	1.77 ± 0.43	0.97 ± 0.21#	0.37 ± 0.15	20.98 ± 2.19#	0.71 ± 0.02*

Groups	Horizontal locomotor activity	Vertical locomotor activity	Hole-board exploration	Self-grooming	Defecation	Total locomotor activity	Asymmetry coefficient
4	18.79 ± 2.03	2.37 ± 0.87	1.87 ± 0.41	1.03 ± 0.28	0.54 ± 0.19	23.88 ± 2.86	0.68 ± 0.04
Second period							
1	12.47 ± 1.41	2.20 ± 0.30	3.53 ± 0.45	1.20 ± 0.22	0.60 ± 0.25	20.07 ± 1.84	0.60 ± 0.03
2	21.00 ± 2.99*	1.93 ± 0.96*	0.87 ± 0.29*	0.73 ± 0.17	0.93 ± 0.25	28.47 ± 4.13*	0.72 ± 0.03*
3	14.69 ± 2.32#	3.24 ± 0.73	3.50 ± 0.95 #	1.33 ± 0.20	0.62 ± 0.19	23.16 ± 3.12	0.63 ± 0.03#
4	17.89 ± 3.72	2.56 ± 0.49	2.89 ± 0.75#	1.89 ± 0.36	1.12 ± 0.32	26.31 ± 3.89	0.68 ± 0.04
Day 14							
Frist period							
1	15.07 ± 1.95	3.27 ± 0.42	1.07 ± 0.51	0.93 ± 0.18	0.13 ± 0.09	18.47 ± 1.24	0.67 ± 0.02
2	19.67 ± 1.63*	2.93 ± 0.35	1.53 ± 0.46	0.53 ± 0.19	0.20 ± 0.11	24.87 ± 2.02*	0.78 ± 0.02*
3	14.65 ± 1.5#	4.13 ± 0.31#	2.02 ± 0.33	0.72 ± 0.17	0.18 ± 0.14	21.11 ± 1.63	0.75 ± 0.04*
4	18.77 ± 2.39*	1.34 ± 0.78	1.19 ± 0.34	1.12 ± 0.19	0.12 ± 0.06	23.56 ± 3.07	0.75 ± 0.03
Second period							
1	18.47 ± 1.99	4.07 ± 0.77	3.87 ± 0.46	1.13 ± 0.19	0.33 ± 0.15	26.92 ± 3.01	0.68 ± 0.03
2	23.80 ± 1.84*	2.93 ± 0.66*	1.60 ± 0.39*	1.07 ± 0.28	0.53 ± 0.14	29.97 ± 1.89	0.80 ± 0.03*
3	16.14 ± 2.17#	4.63 ± 0.54#	3.39 ± 0.72#	2.89 ± 0.24	0.43 ± 0.19	26.75 ± 3.12	0.57 ± 0.04#
4	24.17 ± 4.56	3.65 ± 0.57	2.89 ± 0.86	1.29 ± 0.37	0.54 ± 0.15	32.56 ± 3.57	0.76 ± 0.04
Day 21							
Frist period							
1	15.11 ± 1.26	2.28 ± 0.22	2.16 ± 0.19	0.92 ± 0.14	0.22 ± 0.08	21.32 ± 1.37	0.70 ± 0.02
2	19.73 ± 1.07*	1.11 ± 0.21*	1.07 ± 0.26*	0.41 ± 0.18*	0.22 ± 0.08	21.65 ± 2.98	0.74± 0.05
3	15.98 ± 1.67#	3.23 ± 0.37#	1.89 ± 0.31	0.72 ± 0.19	0.51 ± 0.19	20.55± 2.35	0.73 ± 0.04
4	16.38 ± 2.36	2.04 ± 0.87	1.79 ± 0.37	0.85 ± 0.24	0.36 ± 0.17	21.03 ± 3.29	0.72 ± 0.04
Second period							
1	11.15 ± 1.51	2.61 ± 0.37	3.32 ± 0.27	1.14 ± 0.36	0.79± 0.23	20.54 ± 1.12	0.63 ± 0.03
2	22.43± 2.69*	1.11 ± 0.26*	0.87 ± 0.35*	0.97 ± 0.18	0.54 ± 0.17	26.88 ± 2.17*	0.75 ± 0.03*
4	14.19 ± 2.67#	2.92 ± 0.44#	2.99 ± 0.32#	1.12 ± 0.12	0.71 ± 0.22	20.31 ± 2.17#	0.63 ± 0.03#
4	16.23 ± 1.89#	1.17 ± 0.12	1.35 ± 0.36	1.24 ± 0.18	0.65 ± 0.23	21.32 ± 1.98#	0.68 ± 0.05

$p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

Disorders of the cognitive function of the CNS were also confirmed by testing mice in relation to the CPAR reproduction. The value of this parameter after long-term ethanol administration was 45.9% and 37.9% of the level of such parameters in intact mice on the 14th and 21st days of observation, respectively (Table 2).

Table 2. CPAR parameters of the intact mice C57BL/6 (1); in C57BL/6 mice with AE (2); with the GRK5 inhibitor administration to mice with AE (3), with the Pan JAK inhibitor administration to mice with AE (3), (M±SEM).

Day	Groups	Proportion of animals with preserved reflex
7	1	0.94 ± 0.07
	2	0.67 ± 0.11*
	3	0.87 ± 0.10
	4	0.64 ± 0.17
14	1	0.87 ± 0.09
	2	0.40 ± 0.13*
	3	0.87 ± 0.10#
	4	0.57 ± 0.14*
21	1	0.87 ± 0.09
	2	0.33 ± 0.13*
	3	0.87 ± 0.09#
	4	0.21 ±0.08*

p < 0.05 in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

The results obtained were completely consistent with the changes characteristic of AE [6,41]. Thus, proper reproduction of the well-known model of ethanol-induced neurodegeneration was noted [30,34].

3.1.1.2. AD model.

Four-week SH administration resulted in the development of practically similar changes in the psychoneurological status of experimental animals. The increase in horizontal locomotor activity and the asymmetry coefficient were recorded during all periods of the experiment (Table 3).

Table 3. Locomotor activity in the "open field" of intact C57BL/6 mice (1); in C57BL/6 mice with AD (2); with the GRK5 inhibitor administration to mice with AD (3), with the Pan JAK inhibitor administration to mice with AD (3), arb. units (M±SEM).

Groups	Horizontal locomotor activity	Vertical locomotor activity	Hole-board exploration	Self-grooming	Defecation	Total locomotor activity	Asymmetry coefficient
Day 3							
Frist period							
1	11.23 ± 1.26	2.35 ± 0.78	1.56 ± 0.47	0.85 ± 0.24	0.56 ± 0.17	16.59 ± 2.36	0.68 ± 0.03
2	15.46 ± 1.78 *	1.75 ± 0.47	2.12 ± 0.58	1.07 ± 0.37	2.36 ± 0.35*	23.13 ± 3.56*	0.79 ± 0.03*
3	10.19 ± 1.17#	2.56 ± 0.63	2.36 ± 0.34	0.68 ± 0.19	1.45 ± 0.23*	16.08 ± 2.44#	0.66 ± 0.03#
4	14.25 ± 2.10	2.03 ± 0.31	1.97 ± 0.39	1.35 ± 0.28	0.89 ± 0.36#	22.68 ± 3.57	0.78 ± 0.04*
Second period							
1	17.25 ± 2.34	3.41 ± 0.62	4.25 ± 0.97	1.58 ± 0.36	0.89 ± 0.21	26.35 ± 3.47	0.67 ± 0.02
2	25.36 ± 2.57*	1.24 ± 0.17*	2.44 ± 0.23*	1.45 ± 0.29	1.03 ± 0.17	32.07 ± 3.26*	0.78 ± 0.04*
3	22.19 ± 2.78	2.36 ± 0.47	3.59 ± 0.48	2.65 ± 0.18	0.65 ± 0.26	29.84 ± 3.66*	0.75 ± 0.03*
4	25.47 ± 4.13	3.07 ± 0.82	3.06 ± 0.41	3.24 ± 0.69*	0.97 ± 0.23	33.17 ± 4.56	0.76 ± 0.04*
Day 7							
Frist period							
1	13.27 ± 2.19	1.87 ± 0.26	4.37 ± 0.98	0.74 ± 0.17	0.98 ± 0.23	21. 39 ± 3.41	0.61 ± 0.03
2	16.08 ± 2.58	2.56 ± 0.48	1.25 ± 0.21*	1.23 ± 0.26	0.65 ± 0.263	22. 44 ± 2.69	0.72 ± 0.02*
3	11.59 ± 1.67#	1.03 ± 0.31	3.26 ± 0.78	0.95 ± 0.22	0.89 ± 0.36	21.28 ± 3.47	0.65 ± 0.03#
4	17.49 ± 2.41	1.59 ± 0.27	2.41 ± 0.54*	1.07 ± 0.27	2.36 ± 0.35*	24.03 ± 3.67	0.66 ± 0.03
Second period							
1	9.87 ± 0.85	2.56 ± 0.52	2.56 ± 0.74	0.0 ± 0.0	1.14 ± 0.21	16.47 ± 1.17	0.62 ± 0.03
2	17.65 ± 2.36*	1.32 ± 0.37	1.17 ± 0.23	1.01 ± 0.11	0.90 ± 0.12	21.78 ± 2.06*	0.80 ± 0.04*
3	14.37 ± 3.49	3.65 ± 0.87#	1.06 ± 0.33	2.34 ± 0.56	1.23 ± 0.18	21.45 ± 3.56	0.68 ± 0.04
4	16.89 ± 2.97 *	3.01 ± 0.44#	1.11 ± 0.35	1.75 ± 0.19	0.74 ± 0.17	22.49 ± 2.75*	0.68 ± 0.04
Day 14							
Frist period							
1	9.27 ± 0.86	2.18 ± 0.24	3.78 ± 0.54	0.23 ± 0.19	0.22 ± 0.14	15.89 ± 1.42	0.61 ± 0.02
2	14.37 ± 1.45*	1.78 ± 0.19	1.22 ± 0.17*	0.56 ± 0.17	0.75 ± 0.16 *	18.35 ± 1.47	0.78 ± 0.03*
3	8.29 ± 1.31#	2.45 ± 0.31	2.90 ± 0.25#	0.44 ± 0.16	0.63 ± 0.19	14.61 ± 2.01	0.58 ± 0.03#
4	12.38 ± 1.76	1.93 ± 0.21	3.11 ± 0.33#	0.58 ± 0.18	0.81 ± 0.19*	18.79 ± 2.34	0.68 ± 0.04
Second period							
1	7.23 ± 0.57	1.23 ± 0.19	1.72 ± 0.21	1.14 ± 0.23	0.45 ± 0.14	11.79 ± 1.06	0.64 ± 0.03
2	12.56 ± 1.41*	1.37 ± 0.24	1.64 ± 0.18	0.0 ± 0.0	0.72 ± 0.18	16.68 ± 2.13*	0.75 ± 0.03*
3	6.32 ± 0.44#	3.24 ± 0.42#	2.34 ± 0.53	2.29 ± 0.36	0.56 ± 0.11	14.03 ± 1.17	0.49 ± 0.03*#
4	11.23 ± 1.46*	2.19 ± 0.17	1.22 ± 0.44	1.78 ± 0.41	0.66 ± 0.23	16.57 ± 2.47*	0.69 ± 0.04
Day 21							
Frist period							
1	12.35 ± 1.54	2.36 ± 0.48	1.03 ± 0.34	1.23 ± 0.18	1.33 ± 0.17	17.94 ± 2.16	0.71 ± 0.04
2	11.25 ± 1.39	1.85 ± 0.37	2.55 ± 0.37	0.98 ± 0.17	0.56 ± 0.09*	18.75 ± 2.56	0.63 ± 0.03
3	14.35 ± 2.07	2.01 ± 0.39	2.16 ± 0.42	1.56 ± 0.20	0.78 ± 0.19	21.30 ± 2.64	0.66 ± 0.03
4	12.36 ± 1.57	2.31 ± 0.55	2.34 ± 0.33	1.64 ± 0.19	0.82 ± 0.23	19.38 ± 3.05	0.63 ± 0.02
Second period							
1	14.57 ± 1.36	4.56 ± 0.78	3.16 ± 0.23	0.0 ± 0.0	0.57 ± 0.14	23.78 ± 3.19	0.61 ± 0.03
2	19.87 ± 1.75*	2.31 ± 0.55*	3.11 ± 0.17	1.03 ± 0.24	1.23 ± 0.26	29.06 ± 3.11	0.66 ± 0.02
3	12.36 ± 1.78#	3.65 ± 0.66	2.25 ± 0.16	1.07 ± 0.12	0.79 ± 0.24	21.34 ± 2.79#	0.57 ± 0.03#
4	20.56 ± 3.07*	3.97 ± 0.54#	3.26 ± 0.27	1.27 ± 0.35	0.85 ± 0.26	29.83 ± 3.66	0.69 ± 0.04

$p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AD.

In addition, the anticholinergic agent caused a decrease in the ability of mice to reproduce the CPAR. Thus, the value of this indicator on the 21st day of observation was only 24.1% of the level of the corresponding parameter in intact mice (Table 4).

Table 4. CPAR parameters of the intact mice C57BL/6 (1); in C57BL/6 mice with AD (2); with the GRK5 inhibitor administration to mice with AD (3), with the Pan JAK inhibitor administration to mice with AD (3), (M±SEM).

Day	Groups	Proportion of animals with preserved reflex
7	1	0.94 ± 0.10
	2	0.60 ± 0.13*
	3	0.87 ± 0.09
	4	0.87 ± 0.09
14	1	0.94 ± 0.10
	2	0.54 ± 0.14*
	3	0.72 ± 0.07#
	4	0.56 ± 0.14*
21	1	0.87 ± 0.09
	2	0.21 ± 0.15*
	3	0.72 ± 0.07#
	4	0.33 ±0.13*

$p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AD.

The obtained data indicated both excessive excitability of the animals (the results of the "open field" test in the first minute of observation) and disorders of cognitive activity (data from the CPAR test and changes in locomotor activity in the 2-3 minutes of observation) [8,29]. Thus, there was a modeling of "persistent" anticholinergic disorders, manifested by damage to the CNS similar to that in AD [8,35].

3.1.2. Progenitor functioning.

3.1.2.1. AE model.

Chronic alcoholization of mice was not accompanied by a statistically significant change in the content of NSC in the SVZ (Figure 1, A1).

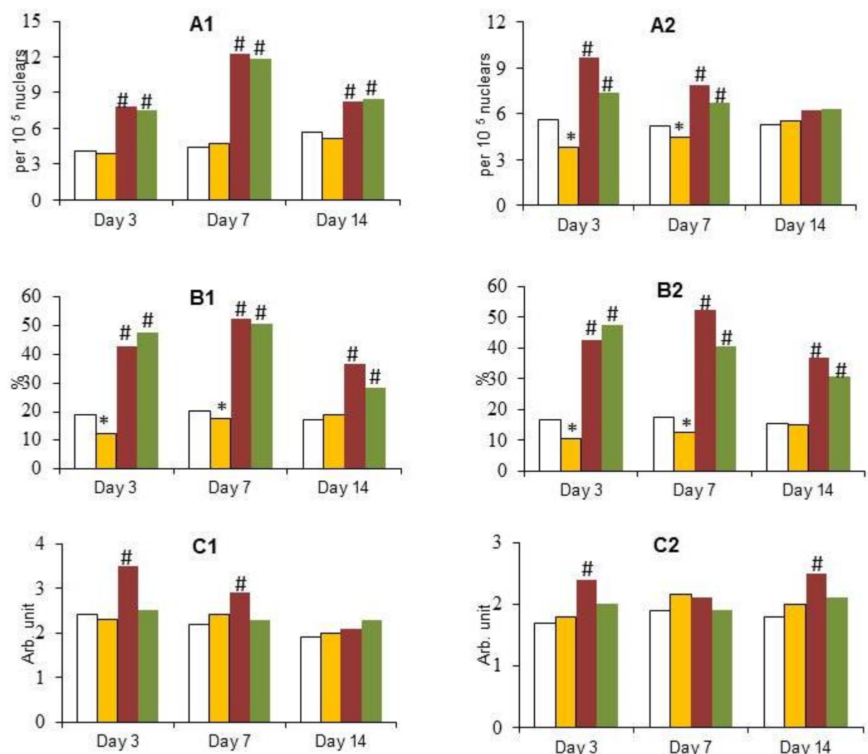

Figure 1. (A) Progenitor amount; **(B)** their proliferative activity; **(C)** differentiation index (**index 1** - NSC, **index 2** - NCP) in intact C57BL/6 mice (white bars); in mice after AE modeling (yellow bars), in mice injected with the GRK5 inhibitor after AE modeling (red bars), in mice injected with the Pan JAK inhibitor after AE modeling (green bars); *- differences at $p < 0.05$.

In this case, a decrease in the number of unipotent neuronal precursors (NCP) was recorded compared to similar indicators in intact mice (up to 67.9% and 86.5% of the AE control on the 3rd and 7th days, respectively) (Figure 1, A2). The decrease in the mitotic activity of NSC and NCP was also noted (Figure 1, B1, B2). The rate of NSC and NCP specialization did not change (Figure 1, C1, C2).

These results were consistent with data on the decrease in the ability of neural tissue progenitors to realize their growth potential during alcoholic neurodegeneration, which is more pronounced in committed precursors [30,31,34].

3.1.2.2. AD model.

Long-term administration of high doses of an anticholinergic agent led to a decrease in the content of both types of progenitor cells in the SVZ (Figure 2, A1, A2). At the same time, a decrease in the number of their actively proliferating forms (up to 80.8% and 72.0% of the AD control on the 3rd and 7th days, respectively) (Figure 2, B1, B2) and the intensity of specialization (Figure 2, C1, C2) were also recorded.

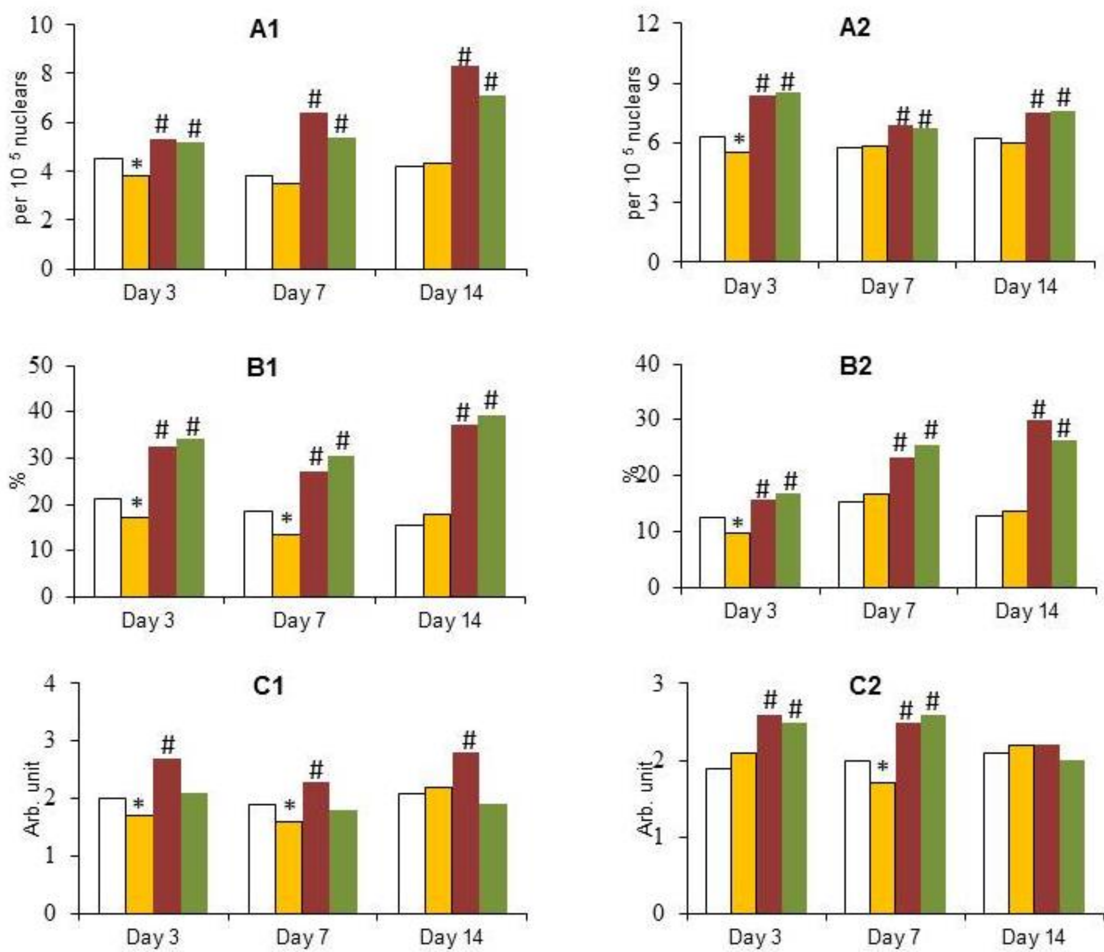

Figure 2. (**A**) Progenitor amount; (**B**) their proliferative activity; (**C**) differentiation index (**index 1** - NSC, **index 2** - NCP) in intact C57BL/6 mice (white bars); in mice after AD modeling (yellow bars), in mice injected with the GRK5 inhibitor after AD modeling (red bars), in mice injected with the Pan JAK inhibitor after AD modeling (green bars); *- differences at $p < 0.05$.

The findings confirmed the data on the participation of acetylcholine receptors (mAchR, nAChR) [42,43] in the disruption of the activity of nervous tissue progenitors under the SH influence [8].

3.2. Blockade of receptor signaling pathways in modeling neurodegenerative conditions.

3.2.1. Effects on cognitive and behavioral disorders.

3.2.1.1. AE model.

HY-136561 administration did not alter the exploratory behavior of AE mice until day 3 after the start of its administration (Table 1). Subsequently (starting from the 7th day), a decrease in horizontal activity, an increase in the number of vertical rearing and peeking into the holes of the "open field" were noted. The most pronounced changes were recorded on the 14th and 21st days of observation. During these periods, the behavioral disturbances characteristic of AE were abolished. At the same time, the decrease in the coefficient of asymmetry of movements was only in the second observation period (2-3 min). This suggests that GRK5 blockade weakly corrected anxiety in alcoholized animals, against the background of a very significant restoration of their cognitive activity [8, 29]. This was confirmed by the results of a study on the reproduction of the developed CPAR in them. The introduction of HY-136561 significantly increased the proportion of animals with a preserved reflex (Table 2).

Pan JAK Inhibitor did not affect the exploratory behavior of mice on days 3–14 after the start of its use (Table 3). During these periods, their locomotor activity corresponded to that of the control animals. Only on the 21st day of the experiment, in the second observation period (2–3 min), was a decrease in the total number of movements noted, and as a result of a decrease in horizontal activity. In addition, Pan JAK Inhibitor did not correct the conditioned reflex activity of experimental animals (Table 4). Moreover, on the 21st day after the start of its administration, a decrease in the level of reproduction of the CPAR was recorded on the 21st day relative to mice with AE that did not receive the pharmacological agent.

3.2.1.2. AD model.

GRK5 Inhibitor significantly reduced the disorders of exploratory behavior in mice (Table 3). Blockade of G-protein-coupled receptor signaling led to normalization of such parameters as horizontal activity of mice and the asymmetry coefficient. It is important that statistically significant differences in these parameters relative to the control were observed in the first observation period (1 min) already on the 3rd day after the start of the administration of the pharmacological agent. This indicates a significant decrease in the anxiety of the experimental animals in this case. This could be associated with the blockade of downstream signaling pathways under the influence of HY-136561: NF-κB- and PI3K-mediated signaling, largely responsible for neuroinflammation developing in response to the administration of an anticholinergic agent [44,45]. The correction of locomotor activity disorders in the second period (2-3 min) of observation (reflecting the restoration of cognitive functions of the CNS [8,30]) was recorded starting from the 14th day. The delay of this consequence of the GRK5 Inhibitor in this case allows us to speak about the participation of the cell renewal system of the “deep reserve” (i.e., progenitors of the nervous tissue [13,17]) in its development. The effect of the GPCR-signaling blocker on the cognitive activity of mice with cholinolytic disorders was confirmed by an increase in the level of reproduction of the CPAR (Table 4).

Unlike HY-136561, Pan JAK Inhibitor had no significant effect on the exploratory behavior of mice (Table 3) or on their conditioned reflex activity (Table 4).

Thus, only blockade of the GPCR-signaling pathway caused correction of disorders of the psychoneurological status characteristic of AE [8,35] and AD [30,34].

3.2.2. Effects on progenitor functioning.

3.2.2.1. AE model.

The introduction of blockers of both GPCR and cytokine receptor signaling significantly stimulated the realization of the growth potential of NSC and NCP in ethanol-induced neurodegeneration. At the same time, the increase in the number of multipotent progenitors in the SVZ using different pharmacological agents was comparable. It reached 200.0%, 261.7% and 159.6% with the introduction of HY-136561 and 192.3%, 251.1% and 163.5% with the introduction of Pan JAK Inhibitor from the level of similar values in untreated mice with AE on the 3rd, 7th, and 14th days, respectively (Figure 1, A1). The increase in the amount of unipotent neuronal precursors in the SVZ was more pronounced after the use of HY-136561 (Figure 1, A2). In both cases (administration of blockers of both receptor signaling), the indicated changes in the progenitor compartments were a consequence of an increase in their proliferative activity (Figure 1, B1, B2). However, HY-136561, unlike Pan JAK Inhibitor, stimulated the process of progenitor specialization. It is obvious that the acceleration of NSC differentiation was at least one of the reasons for the more pronounced increase in the content of NCP in the SVZ with GRK5 inactivation compared to that with JAKs blockade (Figure 1, C1, C2).

3.2.2.2. AD model.

The use of inhibitors of both signaling pathways was accompanied by identical changes. HY-136561 and Pan JAK Inhibitor increased the representation of NSC and NCP in the SVZ of mice after modeling brain pathology (with maxima: NSC — up to 193.0% and 165.1% on the 14th day; NCP — up to 152.7% and 154.5% on the 3rd day from the corresponding control values with blockade of GRK5 and JAKs) (Figure 2, A1, A2). At the same time, a comparable increase in the mitotic activity of progenitors (Figure 2, B1, B2) and the intensity of their specialization was also recorded (Figure 2, C1, C2).

Thus, blockade of GPCR and cytokine receptor signaling had almost the same effect on the functioning of resident progenitors of the brain under conditions of modeling neurodegenerative conditions of various genesis. In both cases, significant stimulation of the realization of the growth potential of NSC and NCP was observed. This was largely consistent with data obtained previously with selective targeting of downstream intracellular second messengers to G-proteins and cytokine receptor signaling in vitro (NF-kB, JNK, AC [7,29,32,33] and JAKs/STAT [31], respectively). It is surprising that these obvious pro-neuroregenerative effects of cytokine receptor pathway blockers are not capable of being realized in the form of effective neurogenesis and stimulation of neuroplasticity in neurodegenerative conditions of various origins. But there are some reasons that can actually explain this phenomenon, which seems paradoxical only in a certain sense.

Firstly, the opposite reaction of nervous tissue progenitors to inhibitors of intracellular signaling molecules JAKs and STATs, depending on the conditions of their vital activity, has been previously shown [12,34,46]. It is known that under the influence of various damaging factors in the NSC and NCP, the pattern of intracellular signaling changes significantly [7,12,17]. In some cases, an inversion of the role of certain intracellular signaling molecules

is observed. With regard specifically to cytokine receptor signaling, in vitro experiments have demonstrated the ability of some JAKs/STAT inhibitors to block the ability of intact (taken from animals without brain pathology) NSC and neural committed precursors to realize their growth potential [34,46]. It is possible that different progenitor populations (stem cells of the SVZ, hippocampus, olfactory bulb, and/or other brain regions [11,47]) also differ in patterns and heterogeneous roles of individual intracellular signaling molecules. In this case, the stimulatory effects of Pan JAK Inhibitor on SVZ progenitors found in the studies simply cannot be relevant to those located in the hippocampus and/or other cambial zones of the brain. Therefore, the integrative effect of this pharmacological action on the deep reserve of regeneration of nervous tissue as a whole did not correspond to the expected results (based on data on cells removed from the SVZ). Moreover, it is known that, for example, in memory processes (as a key element of cognition), the hippocampus plays a decisive role [48].

Secondly, this "paradox" may be associated with the specificity of other aspects of neurogenesis and neuroplasticity (not directly related to the progenitor proliferation). It is possible that under conditions of neurodegeneration, mitoses of neural cells induced by blockade of cytokine receptor signaling cause such a reorganization of the structural and functional organization of nervous tissue that is not capable of being realized in the form of therapeutic effects [31]. That is, in this case, neurogenesis is simply ineffective [49]. In addition, this may be due to the negative impact of this targeting on the functions of neuroglia and/or mature neurons [17,13,31].

The situation is different with GPCR signaling (Figure 3). The results of experiments have shown that inactivation of GRK (at least GRK5) in conditions of neurodegeneration causes stimulation of the functions of nervous tissue progenitors associated with the restoration of cognitive activity of the CNS. At the same time, the registration of a pronounced anti-anxiety effect after the first HY-136561 administration also indicates a direct effect of G-protein blockade on psychoemotional status. Obviously, this could not be associated with neurogenesis. Obviously, this could not be related to neurogenesis. The effects of structural and functional restoration through activation of the cell renewal system require significant time for the specialization of newly formed cells and their inclusion in existing neuronal ensembles and/or the formation of such de novo [7,31,50]. Therefore, it appears that the effects observed early after the start of administration of the pharmacological agent were associated with the correction of the direct involvement of GPCR signaling, at least in providing such an emotional reaction as irritability [51]. One of the mechanisms for this could be the anti-inflammatory effect of the GRK5 inhibitor, which blocks the implementation of downstream NF-kB-, PI3K-dependent, and other pro-inflammatory pathways [32,52]. However, given the ubiquity of GPCR and the widest range of cellular functions regulated through them (neurotransmission, cellular metabolism, secretion, immune responses, etc.), the mechanism for the development of the identified phenomena combined many different pleiotropic pharmacological effects [25-28].

The identified delayed effects on the psychoneurological status, which persisted for a long time after the end of the administration of the GPCR signaling blocker, were obviously associated with neuroplasticity and the implementation of deep reserve mechanisms of regeneration [2,13,17]. It is very important that the above-mentioned functional heterogeneity of cytokine receptor signals (depending on the localization of precursors in different cambial zones of the brain) in relation to the role of GPCR for regenerative-competent cells was not

observed. At least, the obtained data do not provide grounds to speak about such a functional compartmentalization of GPCR signaling for different populations of neural progenitors.

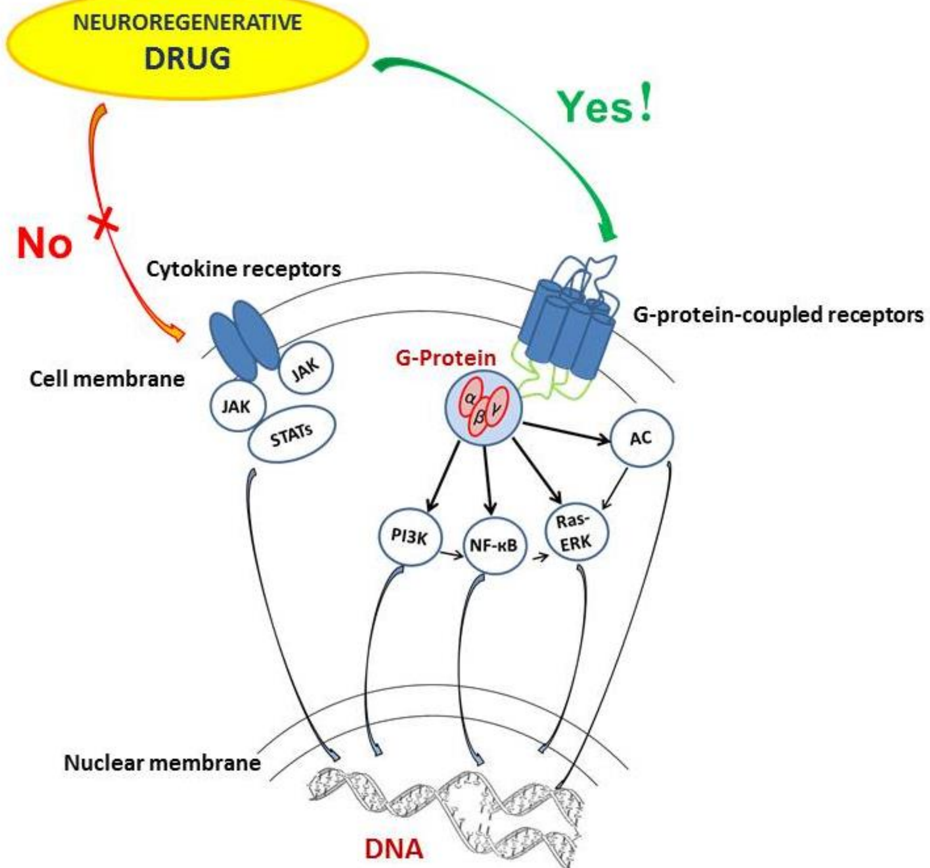

Figure 3. Schematic of GPCR and cytokine receptor signaling for targeting by neuroregenerative drugs.

4. Conclusions

The results of the studies indicate the potential of using GPCR (rather than cytokine receptors) as pharmacological targets for potential drugs with neuroregenerative activity for the treatment of AE and AD. In this case, one should undoubtedly take into account the heterogeneity and multiplicity of G-protein signaling pathways and their varieties [25-28]. Targeting different types of these serine/threonine kinases or even their different subunits (α-, β-, γ-subunits) or isoforms of these subunits [28] can significantly alter the nature of the pharmacological effects on both neural progenitors and CNS dysfunction in neurodegenerative conditions. In addition, when developing neuroregenerative drugs that affect GPCR signaling, it is necessary to take into account the known data on their possible participation in the tumor transformation of cells [53]. Although this danger, based on the available information, is lower than that of the same cytokine receptor signaling pathway [54].

Author Contributions

Conceptualization, G.N.Z; methodology, G.N.Z.; investigation and data collection, L.A.M., T.Yu.P, S.E.V., A.V.C., P.O.Z.; writing—original draft preparation, G.N.Z.; writing—review and editing, G.N.Z.; visualization, G.N.Z.; project administration G.N.Z. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was carried out in accordance with the principles of the humane treatment of animals (EU Directive 2010/63/EU). Permission was previously obtained from the local ethics committee of the Goldberg Research Institute of Pharmacology and Regenerative Medicine (protocol IACUC-2023-04/18).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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