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Acute and repeated treatment with the 5-HT$_7$ receptor antagonist SB 269970 induces functional desensitization of 5-HT$_7$ receptors in rat hippocampus

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Abstract:
Background: SB 269970, a 5-HT$_7$ receptor antagonist may produce a faster antidepressant-like effect in animal models, than do antidepressant drugs, e.g., imipramine. The present work was aimed at examining the effect of single and repeated (14 days) administration of SB 269970 on the 5-HT$_7$ receptor in the hippocampus.
Methods: The reactivity of 5-HT$_7$ receptors was determined using 5-carboxamidotryptamine (5-CT), which increased the bursting frequency of spontaneous epileptiform activity in hippocampal slices. Additionally, the effects of SB 269970 administration on the affinity and density of 5-HT$_7$ receptors were investigated using [^{3}H]-SB 269970 and the influence of SB 269970 and imipramine on mRNA expression levels of Gα_s and Gα_{12} mRNA were studied using RT-qPCR.
Results: Acute and repeated treatment with SB 269970 led to attenuation of the excitatory effects of activation of 5-HT$_7$ receptors. Neither single nor repeated administration of SB 269970 changed the mean affinity of 5-HT$_7$ receptors for [^{3}H]-SB 269970. Repeated, but not single, administration of SB 269970 decreased the maximum density of [^{3}H]-SB 269970 binding sites. While administration of imipramine did not change the expression of mRNAs for Gα_s and Gα_{12} proteins after both single and repeated administration of SB 269970, a reduction in Gα_s and Gα_{12} mRNA expression levels was evident.
Conclusions: These findings indicate that even single administration of SB269970 induces functional desensitization of the 5-HT$_7$ receptor system, which precedes changes in the receptor density. This mechanism may be responsible for the rapid antidepressant-like effect of the 5-HT$_7$ antagonist in animal models.

Key words:
5-carboxamidotryptamine, adaptive changes, epileptiform activity, hippocampal slice, imipramine, SB 269970

Introduction

Serotonin (5-hydroxytryptamine, 5-HT), which acts as a neurotransmitter and/or a neuromodulator, is involved in a wide spectrum of physiological processes including sleep, cognition, sensory perception, motor activity, temperature regulation, appetite, hormone secretion, nociception, and sexual behavior (reviewed in: [30]). Dysfunctions of the serotonergic system are thought to be involved in the pathomechanism of depressive disorders. Besides other structures, the hippocampus plays an important role as a target for anti-

depressant and anxiolytic drugs [72] (reviewed in: [40, 47]). It has been suggested that a common result of different types of antidepressant therapies is an enhancement of 5-HT neurotransmission within the hippocampus (reviewed in: [9, 19, 27]).

The cellular effects of 5-HT are mediated by up to 14 distinct membrane receptor subtypes that may be expressed in various amounts in single neurons (reviewed in: [28, 29, 55]). Such a diversity permits the occurrence of different effects of 5-HT, including both, inhibitory and excitatory influence on neuronal networks. These mechanisms allow 5-HT to remodel neuronal excitability in a variety of cell types and neuronal circuits in a functionally appropriate manner. In the hippocampus, the most prominent modulatory effect of 5-HT is a 5-HT$_{1A}$ receptor-mediated reduction of the excitability of pyramidal cells [1]. Another 5-HT receptor subtype which effectively modulates neuronal activity is the 5-HT$_4$ receptor whose activation increases excitability of hippocampal pyramidal cells [12, 15]. Adaptive modifications of serotonergic mechanisms modulating the functions of forebrain structures provide an effective mechanism of antidepressant therapies (reviewed in: [8, 36, 39]). In rats, repeated administration of tricyclic antidepressants (TCAs) enhances the inhibitory effect of 5-HT$_{1A}$ receptor activation on the excitability of hippocampal pyramidal neurons [6, 13, 17, 35]. Adaptive changes induced by treatment with the TCA imipramine in rat hippocampus involve attenuation of the excitatory effect of 5-HT$_4$ receptor activation [7, 74]. Repeated administration of selective serotonin reuptake inhibitors (SSRIs) reduces the effectiveness of hippocampal 5-HT$_4$ receptor activation as well; however, at variance with the effects of TCA, the sensitivity of hippocampal 5-HT$_{1A}$ receptors remains unchanged after treatment with SSRIs [7, 13, 14, 62] (reviewed in: [27]).

The 5-HT$_7$ receptor is the latest 5-HT receptor subtype to be identified [4, 54]. In the brain, the 5-HT$_7$ receptor is predominantly expressed in the thalamus, hippocampus, hypothalamus [48] and raphe nuclei [37]. This receptor has been implicated in mood regulation, circadian rhythmicity and sleep, the disturbances of which are evident in the course of affective disorders (reviewed in: [26, 61]). It has been well established that neuronal 5-HT$_7$ receptors activate adenylate cyclase through Gα_s protein [34, 56]. Interestingly, it has been shown that activation of the 5-HT$_{7A}$ receptor stimulates AC1 and AC8 Ca^{2+}/calmodulin-dependent isoforms of adenylate cyclase which are insensitive to Gα_s *in vivo* [3]. Moreover, it has been found that 5-HT$_7$ receptors may also activate Gα_{12} protein [32]. On a cellular level, activation of the 5-HT$_7$ receptor decreases potassium conductances and increases the hyperpolarization-activated current I_h, and thus enhances the excitability of hippocampal pyramidal cells [2, 5, 65]. All these effects contribute to the 5-HT$_7$ receptor-mediated facilitation of hippocampal population spikes *in vivo* [38], as well as to the enhancement of epileptiform activity in disinhibited hippocampal slices *in vitro* [20, 49, 64].

It has been suggested that the modification of 5-HT$_7$ receptor activity resulting from chronic treatment with antidepressants may represent a mechanism underlying the therapeutic effect of these drugs [57] (reviewed in: [24, 26]). It is noteworthy that several psychotropic drugs exhibit high affinity for 5-HT$_7$ receptors [50, 52, 56, 59] (reviewed in: [33]). It has also been shown that certain antidepressants may exert some effects by acting directly on the 5-HT$_7$ receptor [46].

Recent studies have demonstrated a synergistic interaction between serotonin receptors antagonists and several antidepressant drugs [53, 67], including the specific 5-HT$_7$ receptor antagonist SB 269970 [11, 70]. In animal models, inactivation or blockade of the 5-HT$_7$ receptor has been shown to induce antidepressant-like behavior [21, 25, 69]. Chronic treatment with antidepressants has also been shown to modify the reactivity of 5-HT$_7$ receptors. The downregulation of the 5-HT$_7$ receptor has been found to take place in rat suprachiasmatic nucleus of the hypothalamus after chronic treatment with TCAs including imipramine, and the SSRI – fluoxetine [46, 58]. Our earlier study indicated attenuation of the effects of activation of rat hippocampal 5-HT$_7$ receptors after treatment with the TCA imipramine and the SSRI citalopram [64]. These findings support the hypothesis that the 5-HT$_7$ receptor may be a target for the action of antidepressants drug. Moreover, blockade of this receptor opens up good possibilities for the treatment of depression [43] (reviewed in: [24, 40, 44]). It is widely known that the response to treatment with conventional antidepressants may be delayed for several weeks.

Previously, our electrophysiological study showed that repeated (14 times), but not single, imipramine administration diminished the reactivity of the 5-HT$_7$ receptor [64]. We also demonstrated that chronic treatment with imipramine modified neither the affinity of 5-HT$_7$ receptors to [^{3}H]-SB 269970 nor the density of those receptors [63].

Recent research has shown that acute administration of SB 269970, a 5-HT_7 receptor antagonist, shortens immobility time in the forced swim test [68]. Moreover, blockade of the 5-HT_7 receptor may produce a faster antidepressant effect than do contemporary antidepressant drugs [42].

Since only the effect of acute administration of SB 269970 has been investigated so far (mainly in behavioral studies), the present work was aimed at examining the effect of acute and prolonged treatment with SB 269970 on the reactivity of the 5-HT_7 receptor in the hippocampus using biochemical and electrophysiological methods. We studied the influence of the blockade of 5-HT_7 receptors on their affinity and density. The 5-HT_7 receptor may be coupled to two different G proteins: $G\alpha_s$ and $G\alpha_{12}$ (reviewed in: [71]). For this reason we also examined the effect of SB 269970 on $G\alpha_s$ and $G\alpha_{12}$ mRNAs expression and compared the impact of 5-HT_7 receptor blockade with the effect of a the classic tricyclic antidepressant (TCA) imipramine.

Materials and Methods

Drugs

5-Carboxamidotryptamine maleate (5-CT), (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl) ethyl]pyrrolidine hydrochloride (SB 269970) and N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-2-pyridinylcyclohexanecarboxamide (WAY 100635) were obtained from Sigma-Aldrich. [^{3}H]-SB 269970 (62.7 Ci/mol) was purchased from Moravek Biochemicals, Brea, CA.

Treatment of animals

The experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, PAS and were carried out in accordance with the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) and the national law. Male Wistar rats, weighing approx. 100 g at the beginning of the experiment, were housed in groups on a controlled light/dark cycle (the light on: 7:00–19:00) and had free access to standard food and tap water. The rats received SB 269970 (1.2 mg/kg, *ip*, dissolved in 0.9% NaCl, volume: 1 ml/kg) either repeatedly for 14 days, or as a single injection. In the latter group, single administration of SB 269970 was preceded by 13 daily injections of 0.9% NaCl. The animals of the control group received 0.9% NaCl once daily for 14 days.

Two separate experimental groups of the animals received imipramine (10 mg/kg, *ip*, dissolved in 0.9% NaCl, volume: 1 ml/kg) once daily for 14 consecutive days, or as a single injection. In the latter group single administration of imipramine was preceded by 13 daily injections of 0.9% NaCl. The animals of the control group for imipramine experiments received 0.9% NaCl once daily for 14 days.

The rats were killed by decapitation two days after the last drug administration; then their brains were removed and used for further analyses.

Slice preparation, electrophysiological recording and data analysis

The brains were immersed in an ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM): NaCl (124), KCl (5), $CaCl_2$ (2.5), $MgSO_4$ (1.3), KH_2PO_4 (1.25), $NaHCO_3$ (24) and D-glucose (10). ACSF was bubbled with a mixture of 95% O_2/5% CO_2. After dissection, the hippocampus was cut into transverse slices (400 μm thick) using a vibrating microtome (Leica, USA).

The slices were left to recover in the holding chamber at 32 ± 0.5°C, for 1–6 h. A single slice was then transferred to the recording chamber of a submerged type and was superfused (1.5 ml/min) with a warmed (32 ± 0.5°C), modified ACSF, in which [NaCl] was raised to 132 mM and [KCl] was lowered to 2 mM, devoid of Mg^{2+} ions. Glass micropipettes filled with 2 M NaCl (1–4 MΩ) were used to record activity from the pyramidal layer of the CA3 area. Spontaneous epileptiform bursts were amplified (Axoprobe 2, Axon Instruments, USA), band-pass filtered (1 Hz – 10 kHz), A/D converted, stored on a PC (1401 interface with Signal 2 software, CED, UK) and analyzed off-line [66]. Drug effects were assessed in terms of changes in bursting frequency (± SEM) and by comparing the average frequency over 6–10 min after the beginning of 5-carboxamidotryptamine maleate (5-CT) application to the baseline values (see Fig. 1). The data from treated and control rats were also compared using a paired *t*-test.

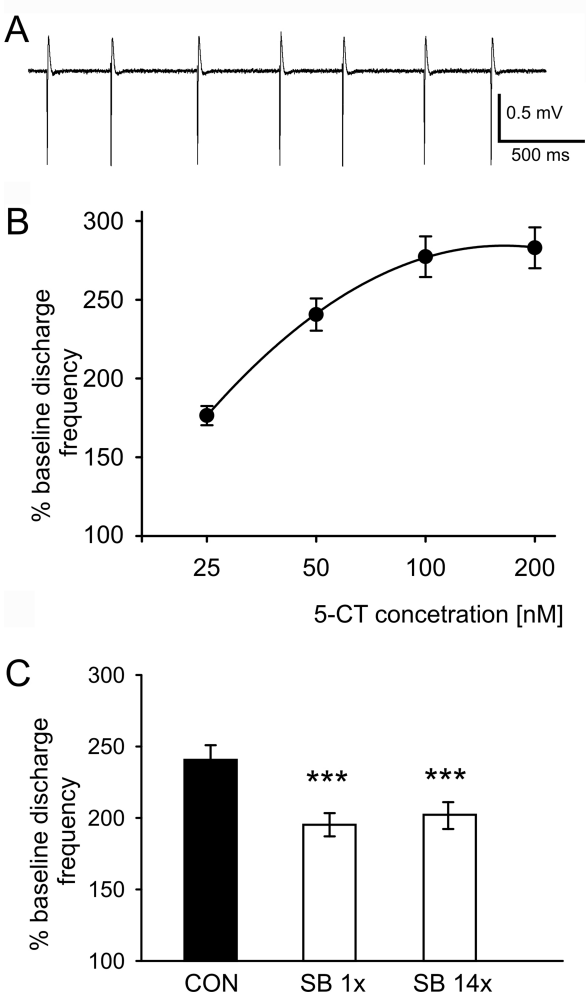

Fig. 1. The influence of administration of the 5-HT$_7$ receptor antagonist SB 269970 on the 5-HT$_7$ receptor-mediated excitatory effect of 5-CT in *ex vivo* hippocampal slices. (**A**) Spontaneous bursting activity recorded in the CA3 area in a representative experiment. (**B**) A dose-response curve for the effect of 5-CT on the bursting activity in control preparations (the mean ± SEM). (**C**) Single (labeled: SB 1×) and repetitive (labeled: SB 14×) administration of SB 269970 resulted in attenuation of the excitatory effect of 50 nM 5-CT compared to slices obtained from untreated rats (CON). *** $p < 0.001$, *t*-test

Membrane preparation and saturation analysis

Rat hippocampi were immediately frozen on dry ice and stored at –80°C. The membranes were prepared according to the method described previously [10] by homogenizing (Ultra Turrax) the tissue in 20 volumes (based on wet weight) of 50 mM Tris-HCl (pH = 7.4 at 37°C). Following centrifugation (50,000 × g, 12 min, 4°C) the pellets were resuspended in the same medium and incubated at 37°C for 15 min. After a further three centrifugation and resuspension steps, the pellets were stored at –80°C for further analysis. Saturation binding assays were performed using [^{3}H]-SB 269970 according to the method described by Thomas et al. [60]. On the day of the experiment, the membranes (approx. 15 mg tissue/tube) were defrosted, suspended in a Tris-HCl buffer (50 mM, pH = 7.4 at 37°C) containing $CaCl_2$ (4 mM), pargyline (0.1 mM) and ascorbic acid (1 mM) and were incubated with [^{3}H]-SB 269970 (eight concentrations within a range of: 0.2–11 nM) for 60 min at 37°C. The non-specific binding was determined using 10 µM 5-HT. The incubation was terminated by passing through Whatman GF/B filters, followed by immediate washing with an ice-cold Tris-HCl buffer. The bound radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy (Beckman L SM 6500). All the assays were performed in triplicate in three separate experiments. The binding data were analyzed using non-linear regression (GraphPad Software Inc., San Diego, USA) generating K_d and B_{max} values.

RNA isolation and RT-qPCR

A frozen hippocampal tissue was placed in the Lysis Buffer (4.5 M guanidine-HCl, 100 mM sodium phosphate, pH 6.6; Roche, Germany) at a volume of 0.4 ml/20 mg of the tissue, and was homogenized by high-speed shaking (30/s) in plastic tubes with stainless steel beads in the TissueLyserII apparatus (Qiagene, USA). Total RNA was purified using a High Pure RNA Tissue Kit (Roche, Germany) according to the manufacturer's protocol. The quantity of RNA was determined spectrophotometrically at 260 nm and 260/280 nm (ND/1000 UV/Vis; Thermo Fisher NanoDrop, USA) and its quality was confirmed by electrophoresis on agarose gel.

A two-step reaction: a reverse transcription (RT) and a quantitative polymerase chain reaction (qPCR) was run in the Chromo4 RealTime PCR Instrument (MJ Research, USA). Five hundred nanograms of total RNA and 2 U of RNase inhibitor (Fermentas, Lithuania) were incubated for 5 min at 65°C and chilled on ice. An RT reaction was performed at a final volume of 20 µl of the reaction mixture containing 1× AMV reverse transcriptase buffer (Finnzymes, Finland), 1 mM deoxynucleotide-3-phosphate mixture (dNTP, Fermentas), 10 µM universal primer oligo (dT), 2 U of the ribonuclease inhibitor (Ribolock, Fermentas, Lithuania), 10 U of AMV reverse transcrip-

tase. The RT reaction was carried out at 42°C for 90 min and was followed by a denaturation step at 70°C for 10 min. The products of RT reaction were amplified using TaqMan probes and primers for the $G\alpha_s$, $G\alpha_{12}$ and hypoxanthine-guanine phosphoribosyltransferase (HPRT) (Assays ID: Rn00569454_m1 and Rn00578965_m1 and Rn01527840_m1, respectively) (Applied Biosystems) and a FastStart Universal Probe Master (Rox) kit (Roche, Germany). Amplification was carried out at a total volume of 10 µl of the reaction mixture containing 1× FastStart Universal Probe Master (Rox) mix (Roche, Germany), 50 ng of cDNA as a PCR template, 900 nM TaqMan forward and reverse primers and 250 nM hydrolysis probe labeled with the fluorescent reporter dye FAM at the 5'-end, and a quenching dye at the 3'-end. The qPCR was run with the following profile: 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min and 40 cycles each at 95°C for 15 s and 60°C for 1 min. The threshold value (Ct) for each sample was set in the exponential phase of PCR, and the ΔΔ Ct method was used for data analysis. HPRT was used as reference gene, and its expression was observed at a constant level in each experimental group of the animals.

Statistical analysis

All the values are given as the mean ± standard error of the mean (SEM). A statistical analysis of the data was performed using Statistica 8.0 software (StatSoft, Tulsa, USA) and a one-way analysis of variance (ANOVA), followed by Fisher's Least Significant Difference (LSD) test, $p < 0.05$ was considered statistically significant. The data from treated and control rats were also compared using a paired *t*-test.

Results

Effect of SB 269970 administration on bursting activity

Epileptiform bursting of a regular frequency occurred within 15–20 min after the placement of slices in a nominally Mg^{2+}-free, modified ACSF. Individual bursting events consisted of an initial, population spike-like waveform (1–2 mV in amplitude), which was followed by a slower, positive-going wave. As reported previously, the application of 5-CT to the ACSF in the presence of WAY 100635 resulted in a dose-dependent, 5-HT_7 receptor-mediated increase in the bursting frequency which reached its maximum between 6–10 min after the start of 5-CT application [49, 64]. Single administration of SB 269970 did not change the mean baseline bursting frequency, which did not differ from that recorded in slices obtained from the control groups of animals receiving the vehicle (Tab. 1). However, repeated administration of SB 269970 resulted in a ca. twofold decrease in the baseline bursting frequency (Tab. 1).

Tab. 1. The influence of single (SB 1×) and repeated (SB 14×) administration of SB 269970 on the mean (± SEM) basal discharge frequency in *ex vivo* hippocampal slices

	Basal discharge frequency [Hz]	*n*
CON	0.116 ± 0.0189	46
SB 1×	0.113 ± 0.0146	30
SB 14×	0.0556 ± 0.0072*	32

* $p < 0.013$, SB 14× *vs.* control

To investigate the effects of SB 269970 administration on the 5-HT_7 receptor-mediated enhancement of the bursting activity, 50 nM 5-CT was applied to the ACSF. As shown in Figure 1C, the magnitude of the 5-CT-induced effect was attenuated in hippocampal slices prepared from the animals receiving single as well as repeated doses of the 5-HT_7 receptor antagonist.

Effect of SB 269970 administration on the affinity and density of 5-HT_7 receptors

Neither single nor repeated administration of SB 269970 changed the mean affinity of 5-HT_7 receptors for [^{3}H]-SB 269970. After single administration, the maximum density (B_{max}) of the receptors also re-

Tab. 2. The influence of single (SB 1×) and repeated (SB 14×) administration of SB 269970 on the mean (± SEM) affinity (pK_d) and the maximum density (B_{max}; ± SEM) of 5-HT_7 receptors in rat hippocampus

	CON	SB 1×	SB 14×
pK_d	0.8.99 ± 0.06	8.78 ± 0.05	9.04 ± 0.06
B_{max} [pM/mg]	0.14 ± 0.003	0.13 ± 0.005	0.09 ± 0.004#

# $p < 0.001$, SB 14× *vs.* control

mained unchanged (Tab. 2). On the contrary, after 14 injections of the 5-HT$_7$ receptor antagonist, the value of B_{max} significantly decreased (Tab. 2).

Effect of imipramine and SB 269970 on G-protein mRNA expression

Neither single nor repeated administration of imipramine induced changes in the expression of mRNAs for the G proteins studied (Figs. 2A$_2$, B$_2$). In contrast, SB 269970 injections caused a statistically significant reduction in Gα_s and Gα_{12} mRNA expression levels. Those effects were evident after both single and repeated administration of the 5-HT$_7$ receptor antagonist (Figs. 2A$_1$, B$_1$).

Discussion

The major finding of this study is that treatment with a specific 5-HT$_7$ receptor antagonist decreases the reactivity of the 5-HT$_7$ receptor, which can bee seen after both single and repeated administration. For the last several years, a number of studies have attempted to evaluate the role of the 5-HT$_7$ receptor in depressive disorders, as well as in the action of antidepressant drugs. It has recently been shown that the blockade of the 5-HT$_7$ receptor synergistically potentiates the effect of clinically used antidepressants in an animal model of depression [70]. Moreover, it has been demonstrated that the antagonist of the 5-HT$_7$ receptor *per se* can exert antidepressive action [42]. The above data suggest that the blockade of the 5-HT$_7$ receptor may play a role in the antidepressive action, and that some antidepressants can exert their therapeutic effect *via* blockade of the 5-HT$_7$ receptor or *via* a decrease in 5-HT$_7$ receptor reactivity. The results of our earlier study showing that chronic treatment with the antidepressants of different classes may decrease the reactivity of the 5-HT$_7$ receptor seem to support this assumption [64].

The present study provides evidence for attenuation of the excitatory effect of activation of the 5-HT$_7$ receptor after treatment with SB 269970. In contrast to several antidepressants tested so far, this effect occurred not only after repeated treatment, but already after single injection of the antagonist. The swift action of SB 269970 on the reactivity of the 5-HT$_7$ receptor is in line with the results obtained by other authors. Mnie-Filali and coworkers [42] found a reduced 5-HT$_{1A}$ and/or 5-HT$_7$ receptor responsiveness in the dorsal raphe nucleus (DRN) after one week of 5-HT$_7$ receptor blockade. To achieve a similar effect for standard antidepressants, at least 21-day treatment is necessary. Thus, the pharmacological blockade of 5-HT$_7$ receptors produces a considerably faster

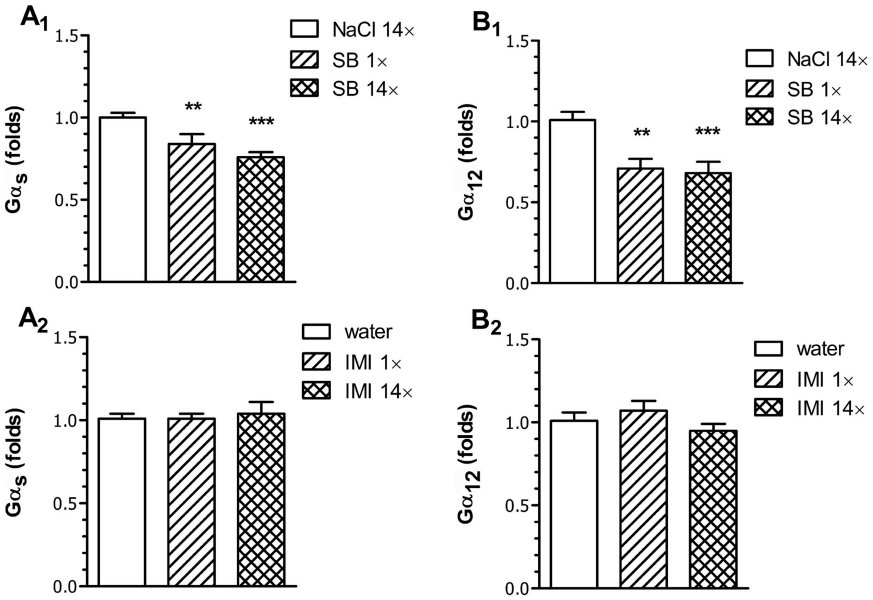

Fig. 2. The influence of treatment with the 5-HT$_7$ receptor antagonist SB 269970 and imipramine on the expression of Gα_s and Gα_{12} mRNA in the hippocampus. (**A**) The Gα_s mRNA expression level after single (1×) or repeated (14×) administration of SB 269970 (labeled SB; **A$_1$**) and imipramine (labeled: IMI; **A$_2$**). (**B**) The Gα_{12} mRNA expression level after single (1×) or repeated (14×) administration of SB 269970 (labeled SB; **B$_1$**) and imipramine (labeled: IMI; **B$_2$**); ** $p < 0.001$, *** $p < 0.0001$ *vs.* vehicle (either NaCl or water); ANOVA, n = 9–10 per group

antidepressant-like response than do most antidepressant drugs [42]. Such a phenomenon may occur as a result of either an increased 5-HT_7 receptor activation or activation of other 5-HT receptors by an elevated extracellular 5-HT level due to the enhanced activity of serotonergic neurons. It has been shown that the blockade of 5-HT_7 receptors increases 5-HT raphe–hippocampus transmission [11]. Enhancement of 5-HT transmission in such projection areas as the dorsal hippocampus after 1 week of SB 269970 administration has also been reported by other researchers [42]. On the other hand, some authors have found that the 5-HT_7 receptor antagonist SB 269970 significantly inhibits 5-HT efflux [51]; however, other investigators have shown that inhibition of 5-HT release is likely to be mediated by 5-HT_7 receptor agonists [22]. The mechanism of control of DRN neuronal activity by 5-HT_7 receptors seems to be indirect, since the regulation of DRN activity *via* the 5-HT_7 receptor is tetrodotoxin-sensitive [23]. This may suggest that 5-HT_7 receptors are not directly localized on 5-HT neurons, but rather on GABAergic and/or glutamatergic ones [23, 45]. It is speculated that the local glutamatergic, GABAergic and serotonergic circuitry in the raphe nuclei forms an excitatory-inhibitory connection by which incoming excitatory signals are converted into an inhibitory output projecting to various brain areas such as, e.g., cerebral cortex, striatum, hippocampus or hypothalamus [23, 45].

Alternatively, the decreased reactivity of hippocampal 5-HT_7 receptors may result from a direct interaction between SB 269970 molecules and the receptor. The molecular mechanism underlying the observed attenuation of the excitatory effect of activation of the 5-HT_7 receptor after treatment with SB 269970 may be related either to the decreased receptor density or modifications in the capacity of the receptor to activate G protein, changes in G protein expression or phosphorylation, or modifications at the level of effectors (reviewed in: [18, 27]). The downregulation of the 5-HT_7 receptor, related to chronic treatment with a variety of antidepressants including imipramine and fluoxetine, was previously found to occur in the suprachiasmatic nucleus of rat hypothalamus [46, 58], where such treatment reduced 5-HT_7 receptor density by approx. 30%, without changing the receptor affinity, though.

As mentioned above, the SB 269970-induced decrease in the reactivity of rat CA1 hippocampal neurons to 5-HT_7 receptor activation may also be related to modifications in the transduction pathway, including changes in the receptor density and/or in the coupling of the receptor to G protein. In fact, our data demonstrate that both single and repeated administration of SB 269970 reduces the level of mRNA of 5-HT_7 receptor coupled G proteins ($G\alpha_s$ and $G\alpha_{12}$). It should be stressed that these changes are specific to the application of the 5-HT_7 antagonist, since imipramine administered once or repeatedly does not alter the level of the mRNAs. We previously showed that repeated administration of imipramine (lasting 14 days) decreased the responsiveness of 5-HT_7 receptors in the CA3 area and that imipramine did not modify the mean basal bursting frequency [64]. However, the present data show that repeated administration of SB 269970 decreases the mean basal bursting frequency, which is lower when compared to the activity recorded in slices obtained from control animals. The most likely explanation of such an effect is reduction of excitatory synaptic transmission. The above results suggest that changes induced by chronic or prolonged administration of SB 269970 depend, at least in part, on mechanisms different from those underlying changes caused by imipramine. It has been demonstrated that activation of 5-HT_7 receptors increases neurite outgrowth in hippocampal neurons [32]. Both $G\alpha_s$ and $G\alpha_{12}$ proteins can regulate cellular morphology by activating different signaling cascades. $G\alpha_s$ protein-mediated morphogenic effects are produced by either modulation of cAMP concentration [16] or direct binding of $G\alpha_s$ protein to the cytoskeleton [73]. The downstream effectors of $G\alpha_{12}$ protein, which mediate changes in the actin cytoskeleton, are members of the Rho family of small GTPases, including RhoA, Rac1 and Cdc42 (reviewed in: [31]). The major functional effects of this pathway, including actin reorganization and the formation of neurite-like protrusions, are mediated by the activation of Cdc42 [32]. It is proposed that activation of the 5-HT_7 receptor by $G\alpha_s$ and $G\alpha_{12}$ proteins may stimulate glutamatergic synaptic transmission by a positive influence on a number of functional synapses. Hence, the prolonged blockade of the 5-HT_7 receptor, resulting in a decreased expression of $G\alpha_s$ and $G\alpha_{12}$ proteins might account for the observed reduction of glutamatergic transmission.

All in all, the current study shows that the blockade of the 5-HT_7 receptor by SB 269970 leads to functional desensitization of the 5-HT_7 receptor system at

a level of its reactivity and effector proteins, although changes in the receptor density occur after chronic treatment with the antagonist only. The phenomenon of 5-HT$_7$ receptor system downregulation may be an important factor in the mechanism of the antidepressant effect of the 5-HT$_7$ antagonist, as well as in the modulation of reactivity of other neurotransmitter systems.

Conflict of interest:
The authors declare no conflict of interest.

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References:

1. Andrade R, Nicoll RA: Pharmacologically distinct actions of serotonin on single pyramidal neurons of the rat hippocampus recorded in vitro. J Physiol, 1987, 394, 99–124.
2. Bacon WL, Beck SG: 5-Hydroxytryptamine7 receptor activation decreases slow afterhyperpolarization amplitude in CA3 hippocampal pyramidal cells. J Pharmacol Exp Ther, 2000, 294, 672–679.
3. Baker LP, Nielsen MD, Impey S, Metcalf MA, Poser SW, Chan G, Obrietan K et al.: Stimulation of type 1 and type 8 Ca^{2+}/calmodulin-sensitive adenylyl cyclases by the Gs-coupled 5-hydroxytryptamine subtype 5-HT$_{7A}$ receptor. J Biol Chem, 1998, 273, 17469–17476.
4. Bard JA, Zgombick J, Adham N, Vaysse P, Branchek TA,Weinshank RL: Cloning of a novel human serotonin receptor (5-HT$_7$) positively linked to adenylate cyclase. J Biol Chem, 1993, 268, 23422–23426.
5. Bickmeyer U, Heine M, Manzke T, Richter DW: Differential modulation of I_h by 5-HT receptors in mouse CA1 hippocampal neurons. Eur J Neurosci, 2002, 16, 209–218.
6. Bijak M, Tokarski K, Czyrak A, Maćkowiak M, Wędzony K: Imipramine increases the 5-HT$_{1A}$-mediated inhibition of hippocampal neurons without changing the 5-HT$_{1A}$ receptor binding. Eur J Pharmacol, 1996, 305, 79–85.
7. Bijak M, Tokarski K, Maj J: Repeated treatment with antidepressant drugs induces subsensitivity to the excitatory effect of 5-HT$_4$ receptor activation in the rat hippocampus. Naunyn-Schmiedeberg's. Arch Pharmacol, 1997, 355, 14–19.
8. Blier P: The pharmacology of putative early-onset antidepressant strategies. Eur Neuropsychopharmacol, 2003, 13, 57–66.
9. Blier P, de Montigny C: Possible serotonergic mechanisms underlying the antidepressant and anti-obsessive-compulsive disorder responses. Biol Psychiatry, 1998, 44, 313–323.
10. Bojarski AJ, Paluchowska MH, Duszyńska B, Kłodzińska A, Tarczyńska E, Chojnacka-Wójcik E: 1-Aryl-4-(4-succinimidobutyl)piperazins and their conformationally constrained analogues: synthesis, binding to serotonin (5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$), a1-adrenergic and dopaminergic D_2 receptors, and in vivo 5-HT$_{1A}$ functional characteristics. Bioorg Med Chem, 2005, 13, 2293–3303.
11. Bonaventure P, Kelly L, Aluisio L, Shelton J, Lord B, Galici R, Miller K et al.: Selective blockade of 5-hydroxytryptamine (5-HT)$_7$ receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. J Pharmacol Exp Ther, 2007, 321, 690–698.
12. Chaput Y, Araneda RC, Andrade R: Pharmacological and functional analysis of a novel serotonin receptor in the rat hippocampus. Eur J Pharmacol, 1990, 182, 441–456.
13. Chaput Y, de Montigny C, Blier P: Presynaptic and post-synaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. Neuropsychopharmacology, 1991, 5, 219–229.
14. Chaput Y, de Montigny C, Blier P: Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. Naunyn Schmiedeberg's Arch Pharmacol, 1986, 333, 342–348.
15. Colino A, Halliwell JV: Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. Nature, 1987, 328, 73–77.
16. Corset V, Nguyen-Ba-Charvet KT, Forcet C, Moyse E, Chédotal A, Mehlen P: Netrin-1-mediated axon outgrowth and cAMP production requires interaction with adenosine A2b receptor. Nature, 2000, 407, 747–750.
17. de Montigny C, Aghajanian GK: Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. Science, 1978, 202, 1303–1306.
18. Donati RJ, Rasenick MM: G protein signalling and the molecular basis of antidepressant action. Life Sci, 2003, 73, 1–17.
19. Dremencov E, Gur E, Lerer B, Newman ME: Effects of chronic antidepressants and electroconvulsive shock on serotonergic neurotransmission in the rat hippocampus. Prog Neuropsychopharmacol Biol Psychiatry, 2003, 27, 729–739.
20. Gill CH, Soffin EM, Hagan JJ, Davies CH: 5-HT$_7$ receptors modulate synchronized network activity in rat hippocampus. Neuropharmacology, 2002, 42, 82–92.
21. Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer MS, Stanton JA, Bromidge F et al.: Genetic knockout and pharmacological blockade studies of the 5-HT$_7$ receptor suggest therapeutic potential in depression. Neuropharmacology, 2005, 48, 492–502.

22. Harsing LG: The pharmacology of the neurochemical transmission in the midbrain raphe nuclei of the rat. Curr Neuropharmacol, 2006, 4, 313–339.
23. Harsing LG, Prauda I, Barkoczy J, Matyus P, Juranyi Z: A 5-HT_7 heteroreceptor-mediated inhibition of [^{3}H]serotonin release in raphe nuclei slices of the rat: evidence for a serotonergic glutamatergic interaction. Neurochem Res, 2004, 29, 1487–1497.
24. Hedlund PB: The 5-HT_7 receptor and disorders of the nervous system: an overview. Psychopharmacology (Berl), 2009, 206, 345–354.
25. Hedlund PB, Huitron-Resendiz S, Henriksen SJ, Sutcliffe JG: 5-HT_7 receptor inhibition and inactivation induce antidepressant-like behavior and sleep pattern. Biol Psychiatry, 2005, 58, 831–837.
26. Hedlund PB, Sutcliffe JG: Functional, molecular and pharmacological advances in 5-HT_7 receptor research. Trends Pharmacol Sci, 2004, 25, 481–486.
27. Hensler JG: Regulation of 5-HT_{1A} receptor function in brain following agonist or antidepressant administration. Life Sci, 2003, 72, 1665–1682.
28. Hoyer D, Hannon JP, Martin GR: Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav, 2002, 71, 533–554.
29. Hoyer D, Martin G: 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology, 1997, 36, 419–428.
30. Jacobs BL, Azmitia EC: Structure and function of the brain serotonin system. Physiol Rev, 1992, 72, 165–229.
31. Jaffe AB, Hall A: Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol, 2005, 21, 247–269.
32. Kvachnina E, Liu G, Dityatev A, Renner U, Dumuis A, Richter DW, Dityateva G et al.: 5-HT_7 receptor is coupled to Gα subunits of heterotrimeric G12-protein to regulate gene transcription and neuronal morphology. J Neurosci, 2005, 25, 7821–7830.
33. López-Rodríguez ML, Benhamú B, Morcillo MJ, Porras E, Lavandera JL, Pardo L: Serotonin 5-HT_7 receptor antagonists. Curr Med Chem Cent Nerv Syst Agents, 2004, 4, 203–214.
34. Lovenberg TW, Baron BM, de Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE et al.: A novel adenylyl cyclase-activating serotonin receptor (5-HT_7) implicated in the regulation of mammalian circadian rhythms. Neuron, 1993, 11, 449–458.
35. Maj J, Bijak M, Dziedzicka-Wasylewska M, Rogoż R, Rogoż Z, Skuza G, Tokarski K: The effects of paroxetine given repeatedly on the 5-HT receptor subpopulations in the rat brain. Psychopharmacology, 1996, 127, 73–82.
36. Mann JJ: Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. Neuropsychopharmacology, 1999, 21, 99–105.
37. Martín-Cora FJ, Pazos A: Autoradiographic distribution of 5-HT_7 receptors in the human brain using [^{3}H]mesulergine: comparison to other mammalian species. Br J Pharmacol, 2004, 141, 92–104.
38. Matsumoto M, Kojima T, Togashi H, Mori K, Ohashi S, Ueno K, Yoshioka M: Differential characteristics of endogenous serotonin-mediated synaptic transmission in the rat hippocampal CA1 and CA3 fields of anaesthetized rats. Naunyn Schmiedebergs Arch Pharmacol, 2002, 366, 570–577.
39. Middlemiss DN, Price GW, Watson JM: Serotonergic targets in depression. Curr Opin Pharmacol, 2002, 2, 18–22.
40. Millan MJ: Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol Ther, 2006, 110, 135–370.
41. Millan MJ: The neurobiology and control of anxious states. Prog Neurobiol, 2003, 70, 83–244.
42. Mnie-Filali O, Faure C, Lambás-Seńas L, Mansari ME, Belblidia H, Gondard E, Etiévant A et al.: Pharmacological blockade of 5-HT_7 receptors as a putative fast acting antidepressant strategy. Neuropsychopharmacology, 2011, 36, 1275–1288.
43. Mnie-Filali O, Lambas-Senas L, Scarna H, Haddjeri N: Therapeutic potential of 5-HT_7 receptors in mood disorders. Curr Drug Targets, 2009, 10, 1109–1117.
44. Mnie-Filali O, Lambás-Senas L, Zimmer L, Haddjeri N: 5-HT_7 receptor antagonists as a new class of antidepressants. Drug News Perspect, 2007, 20, 613–618.
45. Monti JM, Leopoldo M, Jantos H: The serotonin 5-HT_7 receptor agonist LP-44 microinjected into the dorsal raphe nucleus suppresses REM sleep in the rat. Behav Brain Res, 2008, 191, 184–189.
46. Mullins UL, Gianutsos G, Eison AS: Effects of antidepressants on 5-HT_7 receptor regulation in the rat hypothalamus. Neuropsychopharmacology, 1999, 21, 352–367.
47. Nestler EJ, Barrot M, Di Leone RJ, Eisch AJ, Gold SJ, Monteggia LM: Neurobiology of depression. Neuron, 2002, 34, 13–25.
48. Neumaier JF, Sexton TJ, Yracheta J, Diaz AM, Brownfield M: Localization of 5-HT_7 receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. J Chem Neuroanat, 2001, 21, 63–73.
49. Pitra P, Tokarski K, Grzegorzewska M, Hess G: Effects of repetitive administration of tianeptine, zinc hydroaspartate and electroconvulsive shock on the reactivity of 5-HT_7 receptors in rat hippocampus. Pharmacol Rep, 2007, 59, 627–635.
50. Purohit A, Smith C, Herrick-Davis K, Teitler M: Stable expression of constitutively activated mutant h5HT_6 and h5HT_7 serotonin receptors: inverse agonist activity of antipsychotic drugs. Psychopharmacology (Berl), 2005, 179, 461–469.
51. Roberts C, Thomas DR, Bate ST, Kew JN: GABAergic modulation of 5-HT_7 receptor-mediated effects on 5-HT efflux in the guinea-pig dorsal raphe nucleus. Neuropharmacology, 2004, 46, 935–941.
52. Roth BL, Craigo SC, Choudhary MS, Uluer A, Monsma FJ Jr, Shen Y, Meltzer HY, Sibley DR: Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther, 1994, 268, 1403–1410.
53. Rogóż Z, Kabziński M: Enhancement of the anti-immobility action of antidepressants by risperidone in the forced swimming test in mice. Pharmacol Rep, 2011, 63, 1533–1538.

54. Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, Arrang JM, Schwartz JC: Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT$_7$) activating cAMP formation. Proc Natl Acad Sci USA, 1993, 90, 8547–8551.
55. Saudou F, Hen R: 5-Hydroxytryptamine receptor subtypes in vertebrates and in vertebrates. Neurochem Int, 1994, 25, 503–532.
56. Shen Y, Monsma FJ Jr, Metcalf MA, Jose PA, Hamblin MW, Sibley DR: Molecular cloning and expression of a 5-hydroxytryptamine7 serotonin receptor subtype. J Biol Chem, 1993, 268, 18200–18204.
57. Shimizu M, Nishida A, Zensho H, Yamawaki S: Chronic antidepressant exposure enhances 5-hydroxytryptamine7 receptor-mediated cyclic adenosine monophosphate accumulation in rat frontocortical astrocytes. J Pharmacol Exp Ther, 1996, 279, 1551–1558.
58. Sleight AJ, Carolo C, Petit N, Zwingelstein C, Bourson A: Identification of 5-hydroxytryptamine7 receptor binding sites in rat hypothalamus: sensitivity to chronic antidepressant treatment. Mol Pharmacol, 1995, 47, 99–103.
59. Smith C, Rahman T, Toohey N, Mazurkiewicz J, Herrick-Davis K, Teitler M: Risperidone irreversibly binds to and inactivates the h5-HT$_7$ serotonin receptor. Mol Pharmacol, 2006, 70, 1264–1270.
60. Thomas DR, Atkinson PJ, Hastie PG, Roberts JC, Middlemiss DN, Price GW: [^{3}H]-SB-269970 radiolabels 5-HT$_7$ receptors in rodent, pig and primate brain tissues. Neuropharmacology, 2002, 42, 74–81.
61. Thomas DR, Hagan JJ: 5-HT$_7$ receptors. Curr Drug Targets CNS Neurol Disord, 2004, 3, 81–90.
62. Tokarski K, Bijak M: Antidepressant-induced adaptive changes in the effects of 5-HT, 5-HT$_{1A}$ and 5-HT$_4$ agonists on the population spike recorded in hippocampal CA1 cells do not involve presynaptic effects on excitatory synaptic transmission. Pol J Pharmacol, 1996, 48, 565–573.
63. Tokarski K, Pitra P, Duszynska B, Hess G: Imipramine counteracts corticosterone-induced alterations in the effects of the activation of 5-HT$_7$ receptors in rat hippocampus. J Physiol Pharmacol, 2009, 60, 83–88.
64. Tokarski K, Zahorodna A, Bobula B, Grzegorzewska M, Pitra P, Hess G: Repeated administration of citalopram and imipramine alters the responsiveness of rat hippocampal circuitry to the activation of 5-HT$_7$ receptors. Eur J Pharmacol, 2005, 524, 60–66.
65. Tokarski K, Zahorodna A, Bobula B, Hess G: 5-HT$_7$ receptors increase the excitability of rat hippocampal CA1 pyramidal neurons. Brain Res, 2003, 993, 230–234.
66. Tokarski K, Zahorodna A, Bobula B, Hess G: Comparison of the effects of 5-HT$_{1A}$ and 5-HT$_4$ receptor activation on field potentials and epileptiform activity in rat hippocampus. Exp Brain Res, 2002, 147, 505–510.
67. Wesołowska A: Potential role of the 5-HT$_6$ receptor in depression and anxiety: an overview of preclinical data. Pharmacol Rep, 2010, 62, 564–577.
68. Wesołowska A, Kowalska M: Influence of serotonin 5-HT$_7$ receptor blockade on the behavioral and neurochemical effects of imipramine in rats. Pharmacol Rep, 2008, 60, 464–474.
69. Wesołowska A, Nikiforuk A, Stachowicz K, Tatarczyńska E: Effect of the selective 5-HT$_7$ receptor antagonist SB 269970 in animal models of anxiety and depression. Neuropharmacology, 2006, 51, 578–586.
70. Wesołowska A, Tatarczyńska E, Nikiforuk A, Chojnacka-Wójcik E: Enhancement of the anti-immobility action of antidepressants by a selective 5-HT$_7$ receptor antagonist in the forced swimming test in mice. Eur J Pharmacol, 2007, 555, 43–47.
71. Vanhoenacker P, Haegeman G, Leysen JE: 5-HT$_7$ receptors: current knowledge and future prospects. Trends Pharmacol Sci, 2000, 21, 70–77.
72. Vythilingam M, Vermetten E, Anderson GM, Luckenbaugh D, Anderson ER, Snow J, Staib LH et al.: Hippocampal volume, memory, and cortisol status in major depressive disorder: effects of treatment. Biol Psychiatry, 2004, 56, 101–112.
73. Yu JZ, Dave RH, Allen JA, Sarma T, Rasenick MM: Cytosolic Gα_s acts as an intracellular messenger to increase microtubule dynamics and promote neurite outgrowth. J Biol Chem, 2009, 284, 10462–10472.
74. Zahorodna A, Tokarski K, Bijak M: Imipramine but not 5-HT$_{1A}$ receptor agonist or neuroleptics induces adaptive changes in hippocampal 5-HT$_{1A}$ and 5-HT$_4$ receptors. Eur J Pharmacol, 2002, 443, 51–57.

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