



# The Effect of Glutathione Deficit During Early Postnatal Brain Development on the Prepulse Inhibition and Monoamine Levels in Brain Structures of Adult Sprague–Dawley Rats

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## Abstract

Recent studies suggest that impaired glutathione synthesis and distorted dopaminergic transmission are important factors in the pathophysiology of schizophrenia. In the present study, on the postnatal days p5–p16, male pups were treated with the inhibitor of glutathione synthesis, L-buthionine-(S,R)- sulfoximine (BSO, 3.8 or 7.6 mmol/kg), and the dopamine uptake inhibitor, GBR 12,909 (5 mg/kg) alone or in combination, and prepulse inhibition of the acoustic startle response (PPI) was evaluated in adult 90-day-old rats. Moreover, the monoamine levels in the cortex and hippocampus of 16-day-old rats or 91-day-old rats were measured. The present results showed that administration of BSO at 3.8 mmol/kg led to a decreasing tendency in PPI for all tested prepulse intensities. In contrast, a combined treatment with BSO in both studied doses and GBR 12,909 did not induce significant deficits in PPI. Moreover, the results of biochemical studies indicated that treatment with BSO or GBR 12,909 alone induced a weak increase in the activity of dopaminergic, serotonergic, and noradrenergic systems in the frontal cortex and hippocampus of 16-day-old rats and 91-day-old rats. However, the combined administration of both substances allowed for maintaining the normal activity of monoaminergic systems in the rat brain. The most significant changes in the functioning of monoaminergic systems were observed in the frontal cortex of 16-day-old rats. Therefore, it seems that the frontal cortex of rat puppies is most sensitive to glutathione deficiencies resulting in increased oxidative stress in neurons. As a result, it can lead to cognitive and memory impairment.

**Keywords** L-Buthionine-(S,R)-sulfoximine (BSO) · GBR 12,909 · Glutathione deficiency · Animal model of schizophrenia · Prepulse inhibition test (PPI) · Brain monoamine levels

## Abbreviations

<b>BSO</b>	L-buthionine-(S,R)-sulfoximine
<b>DA</b>	Dopamine
<b>DOPAC</b>	3,4-Dihydroxyphenylacetic acid
<b>FCX</b>	Frontal cortex

<b>GBR 129,091</b>	-[2-[Bis-4(fluorophenyl)methoxy]ethyl]-4-3-(3-phenylpropyl)piperazine hydrochloride
<b>5-HIAA</b>	5-Hydroxyindoleacetic acid
<b>HPLC</b>	High-performance liquid chromatography
<b>5-HT</b>	Serotonin
<b>HVA</b>	Homovanillic acid
<b>2-MT</b>	3-Methoxytyramine
<b>NA</b>	Noradrenaline
<b>NM</b>	Normetanephrine
<b>ODS</b>	Osteogenic disorder Shionogi
<b>PPI</b>	Sensorimotor gating, prepulse inhibition test

## Highlights

1. Multiple injections of BSO alone and in combination with GBR 12,909 do not disturb sensorimotor gating.
2. Treatment with GBR 12,909 causes an increase in dopaminergic and serotonergic system activity in the frontal cortex and hippocampus of rats on p 16 and p 91.
3. Treatment with BSO causes an increase in dopaminergic and noradrenergic system activity in the frontal cortex and hippocampus of rats on p 91.
4. Combined administration of BSO and GBR 12,909 maintains the normal activity of the monoaminergic systems in the rat brain.

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## Introduction

Schizophrenia is a chronic and severe mental illness affecting approximately 0.5–1% of the world population (Lewis and Lieberman 2000; Goldner et al. 2002). It

develops progressively, often remaining undetected during childhood and adolescence, with the first episodes of psychosis that appear in early adulthood. The symptoms of this disorder are well characterized and divided into three main categories: positive symptoms (delusions, hallucinations, thought disorder, and incoherence), negative symptoms (lack of motivation and deficits in social function, flat affect), and cognitive deficits (such as memory, attention and executive functions) which are also recognized as a fundamental feature of this illness (Tamminga and Holcomb 2005).

According to the dopamine (DA) hypothesis of schizophrenia, it is postulated that the hypofunction of the cortical and prefrontal DA systems contributes to negative symptoms and cognitive deficits and that the subcortical and limbic DA system hyperactivity causes positive symptoms of schizophrenia (Davis et al. 1991).

In therapy of schizophrenia, typical antipsychotic drugs (i.e., antagonists of dopamine D<sub>2</sub> receptors) mainly inhibit only the positive symptoms (Pearlson 2000; Nuechterlein et al. 2004). In contrast to typical antipsychotics, atypical antipsychotic drugs partially alleviate the negative symptoms and improve the impaired cognitive functions (Schotte et al. 1996; Geyer and Ellenbroek 2003).

Moreover, clinical and preclinical studies have indicated that schizophrenia is associated with neurodevelopmental, structural, and functional brain alterations. The etiology of this disease suggests that both structural and functional abnormalities could be a consequence of multiple interactions between genetic and environmental factors during development (van Os et al. 2008) that set off a cascade of events extending into adulthood (Rapoport and Gogtay 2011). The symptoms of schizophrenia are well characterized, but the mechanism underlying the pathogenesis of the disease still remains unknown. It has been proposed that oxidative stress as a consequence of the aberrant redox control is an attractive hypothesis for explanation, at least partially, of the pathophysiology of schizophrenia (Do et al. 2009; Bitanirwe and Woo 2011; Yao and Keshavan 2011).

Several studies have shown that the level of glutathione, the major antioxidant and redox regulator, is decreased in the cerebrospinal fluid and medial frontal cortex of drug-naïve schizophrenic patients (Do et al. 2000) as well as in the post-mortem striatum (Yao et al. 2006) and prefrontal cortex of those treated earlier with antipsychotic drugs (Gawryluk et al. 2011).

In experimental animals, the effect of the brain glutathione deficit during early postnatal brain development was studied in animal models in adulthood (Rougemont et al. 2002; Castagné et al. 2004a, b).

Those studies showed that chronic combined treatment of osteogenic disorder Shionogi (ODS) mutant rats, which, like humans, cannot synthesize ascorbic acid, with the inhibitor

of glutathione synthesis, L-butionine-(*S,R*)-sulfoximine (BSO), and dopamine reuptake inhibitor, GBR 12,909, during early postnatal life induced schizophrenia-like memory deficits assessed in the novel object recognition test during adulthood. Chronic BSO administration causes glutathione deficiency (Cabungcal et al. 2007). Moreover, treatment with GBR 12,909 reduced glutathione contents and increased lipid peroxidation in the frontal cortex and hippocampus (De Queiroz et al. 2018).

Correspondingly, treatment of ODS and Wistar rats during early postnatal life with BSO alone evoked impairment of some cognitive functions assessed in adulthood in the radial maze with controlled olfactory cues (Cabungcal et al. 2007). In addition, our recently study indicated that in male Sprague–Dawley rat inhibition of glutathione synthesis by repeated treatment with BSO together with DA reuptake inhibitor GBR 12,909 in early postnatal development induced long-term deficits corresponding to schizophrenia-like behavior evaluated in the social interaction test, novel object recognition test, and enhanced the locomotor hyperactivity induced by amphetamine. The above-mentioned behavioral tests are widely used to study some negative, cognitive symptoms, and positive symptoms of schizophrenia. On the other hand, repeated treatment with the inhibitor of glutathione synthesis BSO alone induced only deficits in some negative and cognitive symptoms of schizophrenia (Górny et al. 2019; Lech et al. 2021).

Glutathione plays an important role in the redox control of various signal transduction pathways and gene expression. Thus, glutathione deficit can alter the function of redox-sensitive proteins implicated in neurotransmission and synaptic plasticity, such as NMDA and GABA<sub>A</sub> receptors as well as calcium-activated K<sup>+</sup> channels. These redox-sensitive proteins could affect dopaminergic, glutamatergic, and GABAergic neurotransmitter systems that are known to be dysfunctional in schizophrenia. All these data seem to confirm the use of BSO and GBR 12,909 as a model substance to induce the neurodevelopmental rat model of schizophrenia (Cabungcal et al. 2006; Lorenc-Koci 2015).

The above data have suggested that the impaired endogenous synthesis of glutathione during early postnatal development plays a significant role in the manifestation of schizophrenic symptoms in adulthood.

In light of the above data, the aim of our study was to evaluate the influence of glutathione deficit during early postnatal development induced by repeated treatment with BSO alone and together with GBR 12,909 on the sensorimotor gating (prepulse inhibition (PPI) test used for evaluating the expression of schizophrenia-like symptoms in adult rats. Moreover, the action of BSO alone and together with GBR 12,909 we evaluated after chronic treatment with both compounds on the monoamine levels in two main brain structures involved in the regulation

of working memory, frontal cortex, and the hippocampus (Arnsten 1997; Pietraszek et al. 2009; Inagaki et al. 2010; Waśnik et al. 2019; Białoń et al. 2020) in 16-day-old rats or 91-day-old rats. The influence of repeated treatment with BSO alone and together with GBR12909 on the monoamine levels in rats has not been studied before.

## Materials and Methods

### Animals and Treatment

A total of 94 male Sprague–Dawley rats (46 of 16-old-days and 48 of 91-day-old rats) were used in the present study.

Pregnant Sprague–Dawley females at embryonic day 16 delivered by Charles River Company (Sulzfeld, Germany) were kept in individual cages under standard laboratory conditions: at room temperature of  $21 \pm 1$  °C with 40–50% humidity on a 12-h light–dark cycle (the lights turned on at 7 a.m.), with free access to standard laboratory chow and tap water. One day after birth, the sex of pups was determined, and only males were left with their mothers to be used in further experimental procedure. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (3.8 and 7.6 mmol/kg, *sc*, daily), a selective inhibitor of glutathione synthesis, and the inhibitor of dopamine reuptake GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. Control pups instead of the BSO and GBR 12,909 were given vehicle. The rats were weighed daily, and the injected quantity was adjusted accordingly to the actual body weight. On postnatal day, p23 rats were weaned and housed in groups of four until p90. Behavioral test (sensorimotor gating) evaluating the expression of schizophrenia-like symptoms was carried out in adulthood (at p90 days of age). The tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected on p16 or p91.

### Drugs

1-[2-[Bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine hydrochloride (GBR 12,909, Abcam Biochemicals, Cambridge, UK) and L-butionine-(*S,R*)-sulfoximine (BSO, Sigma-Aldrich, Saint Louis, MO, USA) were dissolved in 0.9% NaCl. The doses of drugs used in the present study were selected based on earlier publications (Castagné et al. 2004a, b; Górný et al. 2019).

### Compliance with Ethical Standards

The experiments were carried out in compliance with the Act on Experiments on Animals of January 21, 2005,

amended on January 15, 2015 (published in Journal of Laws no 23/2015 item 266, Poland), and according to the Directive of the European Parliament and of the Council of Europe 2010/63/EU of 23 September 2010 on the protection of animals used for scientific purposes. They received also an approval of the Local Ethics Committee at the Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków (permission no 3/2018 of 11 January 2018). All efforts were made to minimize the number and suffering of animals used.

### Sensorimotor Gating (Prepulse Inhibition Test, PPI)

The sensorimotor gating was measured at postnatal day 90 (p90). The number of rats in each group (Vehicle, BSO, BSO + GBR 12909, and GBR 12909) was seven to eight. The PPI procedure was performed according to previously published studies (Wędzony et al. 2000, 2008; Chamera et al. 2020). The startle apparatus (SR-LAB, San Diego Instruments, CA, USA) consisted of 8 individual, sound-proof, ventilated chambers containing a single plexiglas cylinder (inner diameter of 9 cm) mounted on the platform. A high-frequency loudspeaker inside each chamber produced both continuous background noise of 65 dB and various acoustic stimuli. The movement of the cylinder caused by the startle response of the animal was transduced into analogue signals by a piezoelectric unit attached to the platform. These signals were then digitized and used in subsequent analyses. Before placing the animals in the chambers, each of them was individually calibrated by an external sensor to display a similar reference stimulus reading. The average startle amplitudes (AVGs) were measured in the registration window of 200 ms. After habituation (5 min, background noise), the animals were randomly subjected to four types of acoustic stimuli. Each experimental trial consisted of either a single pulse [intensity: 120 dB, duration: 40 ms, (P)] or a pulse preceded by a prepulse at one out of three intensities [70, 75, 80 dB; duration: 20 ms; (PP)] applied 80 ms before the pulse. During each experimental session, 20 trials of each type were presented with an interstimulus interval of 20 s. The AVG values were recorded, and the percentage of PPI (PPI%) induced by each prepulse intensity was calculated as  $PPI\% = [(P - PP)/P] \times 100\%$ .

### Biochemical Analysis of Concentrations of Monoamines and Their Metabolites

Sixteen-day-old rats, 4 h after last dose of drug administration, or 91-day-old rats were decapitated. The frontal cortex and hippocampus were dissected and frozen on solid CO<sub>2</sub> (–70 °C) and stored until biochemical assays. Dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and final metabolite, homovanillic acid (HVA); serotonin (5-HT) and its

metabolite 5-hydroxyindoleacetic acid (5-HIAA); and noradrenaline (NA) and its metabolite normetanephrine (NM) were assayed by means of high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatograph (HP 1050; Hewlett-Packard, Golden, CO, USA) was equipped with C18 columns. The procedure of sample preparation is based on our previous protocol (Wąsik et al. 2019).

## Statistical Analysis

The results from behavioral and biochemical experiments were analyzed by means of a two-way ANOVA followed, when appropriate, by the Duncan's post hoc test. The results were considered statistically significant when  $p < 0.05$ .

## Results

Figure 1 presents the timeline of the general protocol used in the present experiments. Our study aimed to evaluate the influence of repeated treated with BSO alone and together with GBR 12909 in the prepulse inhibition test (PPI) and on the monoamine levels in the frontal cortex and hippocampus in 16-day-old rats or 91-day-old rats.

### The Effects of Combined Administration of BSO (3.8 or 7.6 mmol/kg) and GBR 12909 (5 mg/kg) on the Sensorimotor Gating (Prepulse Inhibition Test, PPI)

Disturbed sensorimotor gating is a multi-modal and cross-species phenomenon observed in schizophrenia (Moriwaki et al. 2009; Mena et al. 2016). In Fig. 2, we presented the impact of repeated treatment with BSO in two doses (3.8 and 7.6 mmol/kg, *sc*) alone and in combination with GBR 12909 (5 mg/kg, *sc*) on prepulse inhibition of the acoustic startle response (PPI) in adult (P90) male rats. Although

the analysis did not reveal statistically significant changes, we noticed that the administration of BSO at 3.8 mmol/kg resulted in a decreasing tendency in PPI for all tested prepulse intensities [70 ( $F_{1,42} = 0.70$ , *ns*), 75 ( $F_{1,42} = 0.61$ , *ns*) and 80 ( $F_{1,42} = 1.45$ , *ns*) dB]. On the contrary, a similar effect was not found for the animals exposed to BSO at 7.6 mmol/kg [70 ( $F_{1,42} = 0.70$ , *ns*), 75 ( $F_{1,42} = 0.61$ , *ns*), and 80 ( $F_{1,42} = 1.45$ , *ns*) dB]. Also, a combined treatment with BSO in both studied doses and GBR12909 did not induce significant deficits in PPI [70 ( $F_{1,42} = 0.40$ , *ns*), 75 ( $F_{1,42} = 2.24$ , *ns*), and 80 ( $F_{1,42} = 0.71$ , *ns*) dB].

## Biochemical Analysis

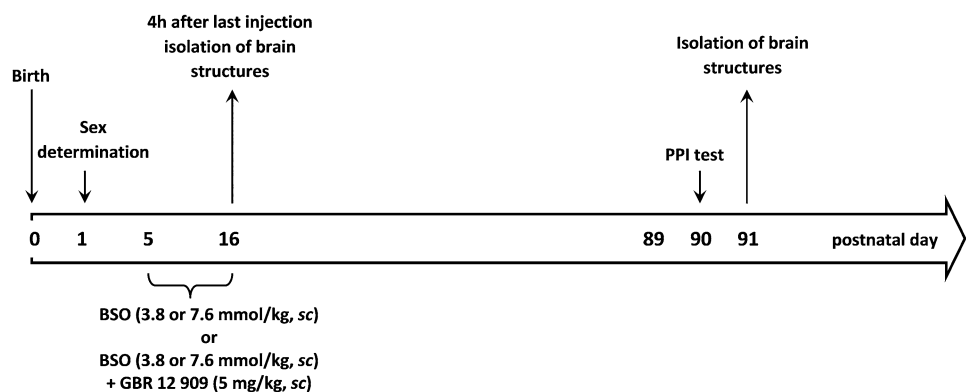
### The Effects of Combined Administration of BSO (3.8 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Frontal Cortex of 16-Day-Old Rats

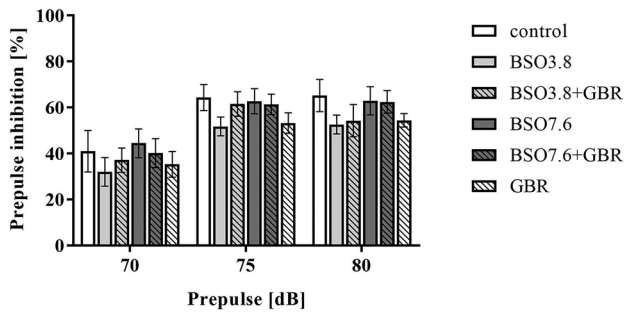
#### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed a significant ( $F_{1,27} = 24.24$ ,  $p < 0.01$ ) effect of treatment 1 (BSO 3.8 mmol) and treatment 2 (GBR 12909 5 mg; ( $F_{1,27} = 12.47$ ,  $p < 0.01$ ) on DA level in the frontal cortex. The same analysis showed a significant effect of interaction of both treatments ( $F_{1,27} = 34.0$ ,  $p < 0.01$ ) on DA level. Post hoc test revealed a significantly increased level of DA after GBR 12909 treatment compared to control ( $p < 0.01$ ). In the combined treatment group, BSO reversed the effect of GBR 12909 and decreased DA amount to the control level ( $p < 0.01$ ) (Table 1).

Two-way ANOVA revealed an insignificant ( $F_{1,27} = 3.35$ , *ns*) effect of treatment 1 and treatment 2 ( $F_{1,27} = 1.80$ , *ns*) on DOPAC level. The effect of interaction of both treatments also was found to be insignificant ( $F_{1,27} = 3.75$ , *ns*) (Table 1).

**Fig. 1** Timeline of the general protocol used in the present experiments





**Fig. 2** The effects of repeated treatment with BSO alone (3.8 and 7.6 mmol/kg, *sc*) and in combination with GBR12909 (5 mg/kg, *sc*) on prepulse inhibition of the acoustic startle response (PPI) in male 90-day-old Sprague–Dawley rats. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (3.8 and 7.6 mmol/kg, *sc*, daily) and GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. Control pups were given vehicle instead of the BSO and GBR 12,909. *n* = 7–8 in each group. The results are presented as the means of percentage of PPI (%PPI) induced by each prepulse intensity ± SEMs. Data were calculated based on the average startle amplitudes (AVGs) using two-way ANOVA with post hoc Duncan test. The statistical outliers were identified using the Grubbs’ test

A statistical analysis showed an insignificant ( $F_{1,27} = 0.06$ , *ns*) effect of treatment 1 and treatment 2 ( $F_{1,27} = 3.38$ , *ns*) on 3-MT level. Interaction of both treatments was found to be insignificant ( $F_{1,27} = 1.8$ , *ns*) (Table 1).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,27} = 0.06$ , *ns*) and a significant effect of treatment 2 ( $F_{1,27} = 13.1$ ,  $p < 0.01$ ) on HVA level in the frontal cortex. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,27} = 0.01$ , *ns*) on HVA level in the frontal cortex (Table 1).

Two-way ANOVA indicated a significant effect of treatment 1 ( $F_{1,27} = 16.89$ ,  $p < 0.01$ ) and an insignificant effect of treatment 2 ( $F_{1,27} = 0.82$ , *ns*) on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 1). The statistical analysis revealed a significant effect of interaction of both treatments ( $F_{1,27} = 24.15$ ,  $p < 0.01$ ). Duncan’s post hoc analysis demonstrated that administration of GBR 12909 decreased the rate of dopamine metabolism ( $p < 0.05$ ) (Table 1), while combined treatment with GBR 12909 and BSO 3.8 mmol reversed the effect of GBR and increased the rate of dopamine metabolism ( $p < 0.01$ ) (Table 1).

**Noradrenaline (NA) and Its Metabolite**

Two-way ANOVA showed an insignificant ( $F_{1,26} = 1.15$ , *ns*) effect of treatment 1 (BSO 3.8 mmol) and treatment 2 (GBR 12909 5 mg; ( $F_{1,26} = 3.99$ , *ns*)) on NA level. At the same time, the effect of interaction of both treatments was significant ( $F_{1,26} = 13.6$ ,  $p < 0.01$ ) on NA level in the frontal cortex. The Duncan’s test revealed a decreased NA amount after combined treatment with BSO and GBR compared with saline ( $p < 0.05$ ) and GBR 12909 ( $p < 0.01$ ) (Table 1).

**Table 1** The effects of combined administration of BSO (3.8 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the frontal cortex of 16-day-old rats

Frontal cortex						
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA
Vehicle	Vehicle	7	63 ± 4	32 ± 2	6 ± 0.7	186 ± 14
Vehicle	GBR 5 mg	8	117 ± 7**	33 ± 3	8 ± .4*	232 ± 12*
BSO 3.8 mmol	Vehicle	8	69 ± 7	32 ± 3	7 ± 0.5	188 ± 15
BSO 3.8 mmol	GBR 5 mg	8	56 ± 2 <sup>++</sup>	24 ± 1 <sup>*+^</sup>	7 ± 1	236 ± 11 <sup>*^</sup>
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA
Vehicle	Vehicle	7	121 ± 3	17 ± 3	47 ± 7	67 ± 1 0
Vehicle	GBR 5 mg	8	131 ± 7	23 ± 1*	67 ± 4*	93 ± 3*
BSO 3.8 mmol	Vehicle	8	137 ± 6	17 ± 2	56 ± 9	80 ± 11
BSO 3.8 mmol	GBR 5 mg	8	103 ± 5 <sup>+++^</sup>	25 ± 1 <sup>**^</sup>	62 ± 4	87 ± 4
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT	
Vehicle	Vehicle	7	303 ± 36	16 ± 2	140 ± 6	
Vehicle	GBR 5 mg	8	204 ± 17*	18 ± 1	140 ± 5	
BSO 3.8 mmol	Vehicle	8	284 ± 23	12 ± 1	134 ± 7	
BSO 3.8 mmol	GBR 5 mg	8	428 ± 22**	25 ± 2**	137 ± 5	

The results are expressed as the means ± SEM (*n* = 7–8 animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan’s post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (3.8 mmol/kg, *sc.*, daily) and GBR 12,909 (5 mg/kg, *sc.*, every second day), alone or in combination. Control pups were given vehicle instead of the BSO and GBR 12,909. The 16-day-old rats were decapitated 4 h after the last dose of drugs, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; + $p < 0.05$ , ++ $p < 0.01$  vs. GBR 12,909-treated group; ^ $p < 0.05$ , ^^ $p < 0.01$  vs. GBR 12,909-treated group



Two-way ANOVA revealed an insignificant ( $F_{1,27}=0.22$ , *ns*) effect of treatment 1 on NM level. The same analysis showed a significant effect of treatment 2 ( $F_{1,27}=13.61$ ,  $p<0.01$ ) on NM amount and insignificant effect of interaction of both treatments ( $F_{1,27}=0.22$ , *ns*). The post hoc test revealed significantly increased level of NM in the GBR group when compared to saline ( $p<0.05$ ). Similar effect was observed in the combined treatment group (BSO + GBR 12909;  $p<0.01$ ) (Table 1).

Two-way ANOVA indicated an insignificant ( $F_{1,26}=0.88$ , *ns*) effect of treatment 1 on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 1). The same analysis showed a significant effect of treatment 2 ( $F_{1,26}=16.73$ ,  $p<0.01$ ) and interaction of both treatments ( $F_{1,26}=8.31$ ,  $p<0.01$ ). Duncan's post hoc analysis demonstrated that combined treatment with GBR 12909 and BSO 3.8 mmol significantly increased the rate of noradrenaline metabolism ( $p<0.01$ ) in the frontal cortex (Table 1).

### Serotonin (5-HT) and Its Metabolite

Two-way ANOVA revealed an insignificant ( $F_{1,25}=0.12$ , *ns*) effect of treatment 1 on 5-HT amount in the frontal cortex. The same analysis showed a significant effect of treatment 2 ( $F_{1,25}=4.57$ ,  $p<0.05$ ) on 5-HT amount and insignificant effect of interaction of both treatments ( $F_{1,25}=1.29$ , *ns*). The post hoc test showed a significant increase in 5-HT level after GBR administration compared to control animals ( $p<0.05$ ) (Table 1).

Two-way ANOVA indicated an insignificant ( $F_{1,27}=0.19$ , *ns*) effect of treatment 1 on 5-HIAA amount. The same analysis showed a significant effect of treatment 2 ( $F_{1,27}=4.44$ ,  $p<0.05$ ) on 5-HIAA amount and insignificant effect of interaction of both treatments ( $F_{1,27}=1.46$ , *ns*). The post hoc test showed a significant increase in 5-HIAA level after GBR 12909 administration compared to control animals ( $p<0.05$ ) (Table 1).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,25}=0.46$ , *ns*), treatment 2 ( $F_{1,25}=0.02$ , *ns*), and interaction of both treatments ( $F_{1,25}=0.09$ , *ns*) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 1).

### The Effects of Combined Administration of BSO (7.6 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Frontal Cortex of 16-Day-Old Rats

#### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed a significant ( $F_{1,26}=5.64$ ,  $p<0.05$ ) effect of treatment 1 (BSO 7.6 mmol) and treatment 2 (GBR 12909 5 mg; ( $F_{1,26}=8.18$ ,  $p<0.01$ )) on the

frontal DA level. The same analysis showed a significant effect of interaction of both treatments ( $F_{1,26}=31.53$ ,  $p<0.01$ ) on DA level. The post hoc test revealed significantly increased level of DA after both GBR 12909 and BSO treatment compared to control ( $p<0.01$  and  $p<0.05$ , respectively). In the combined treatment group, BSO reversed the effect of GBR and decreased DA amount to the control level ( $p<0.01$ ) (Table 2).

Two-way ANOVA revealed an insignificant ( $F_{1,26}=0.001$ , *ns*) effect of treatment 1 and treatment 2 ( $F_{1,26}=2.58$ , *ns*) on DOPAC level. In contrast, the effect of interaction of both treatments was significant ( $F_{1,26}=5.03$ ,  $p<0.05$ ). The Duncan's test revealed that combined treatment with both compounds (BSO + GBR) decreased DOPAC level in comparison with the BSO group ( $p<0.05$ ) (Table 2).

A statistical analysis showed a significant ( $F_{1,26}=4.57$ ,  $p<0.05$ ) effect of treatment 1 and insignificant effect of treatment 2 ( $F_{1,26}=0.34$ , *ns*) on 3-MT level in the frontal cortex. At the same time, interaction of both treatments was significant ( $F_{1,26}=7.57$ ,  $p<0.01$ ) (Table 2). The post hoc test showed a significant increase in 3-MT level after both BSO and GBR 12909 administration compared to control animals ( $p<0.01$  and  $p<0.05$ ), respectively (Table 2).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,26}=0.04$ , *ns*) and a significant effect of treatment 2 ( $F_{1,26}=17.13$ ,  $p<0.01$ ) on HVA level in the frontal cortex. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,26}=0.005$ , *ns*) on HVA level in the frontal cortex (Table 2). The Duncan's test revealed a significant elevation of HVA amount after administration of GBR 12909 alone and in combination with BSO ( $p<0.01$  and  $p<0.05$ ), respectively (Table 2).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,26}=2.33$ , *ns*) and treatment 2 ( $F_{1,26}=0.2$ , *ns*) on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 2). The statistical analysis revealed a significant effect of interaction of both treatments ( $F_{1,26}=24.59$ ,  $p<0.01$ ). Duncan's post hoc analysis demonstrated that administration of GBR 12909 decreased the rate of dopamine metabolism ( $p<0.01$ ). Similar effect was observed after administration of BSO 7.6 mmol ( $p<0.05$ ) (Table 2).

#### Noradrenaline (NA) and Its Metabolite

Two-way ANOVA showed an insignificant ( $F_{1,25}=3.49$ , *ns*) effect of treatment 1 (BSO 7.6 mmol) and treatment 2 (GBR 12909 5 mg; ( $F_{1,25}=0.41$ , *ns*)) on NA level. Similarly, the effect of interaction of both treatments on NA level in the frontal cortex was insignificant ( $F_{1,25}=1.59$ , *ns*) (Table 2).

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,26}=0.31$ , *ns*), treatment 2 ( $F_{1,26}=0.52$ , *ns*),

**Table 2** The effects of combined administration of BSO (7.6 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the frontal cortex of 16-day-old rats

Frontal cortex							
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA	
Vehicle	Vehicle	7	63 ± 4	32 ± 2	6 ± 0.7	186 ± 14	
Vehicle	GBR 5 mg	8	117 ± 7**	33 ± 3	8 ± 0.4*	232 ± 12**	
BSO 7.6 mmol	Vehicle	7	84 ± 9*	37 ± 3	9 ± 0.9**	184 ± 8	
BSO 7.6 mmol	GBR 5 mg	8	67 ± 3 <sup>++</sup>	28 ± 1 <sup>^</sup>	8 ± 0.6	229 ± 8* <sup>^</sup>	
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA	
Vehicle	Vehicle	7	121 ± 3	17 ± 3	47 ± 7	67 ± 10	
Vehicle	GBR 5 mg	8	131 ± 7	23 ± 1	67 ± 4*	93 ± 3	
BSO 7.6 mmol	Vehicle	7	118 ± 5	23 ± 3	53 ± 7	71 ± 8	
BSO 7.6 mmol	GBR 5 mg	8	115 ± 4	20 ± 1	66 ± 3*	90 ± 2	
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT		
Vehicle	Vehicle	7	303 ± 36	16 ± 2	140 ± 6		
Vehicle	GBR 5 mg	8	204 ± 17**	18 ± 1	140 ± 5		
BSO 7.6 mmol	Vehicle	7	228 ± 13*	20 ± 3	136 ± 5		
BSO 7.6 mmol	GBR 5 mg	8	347 ± 18	17 ± 1	136 ± 5		

The results are expressed as the means ± SEM ( $n=7-8$  animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan's post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (7.6 mmol/kg, *sc*, daily) and GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. The 16-day-old rats were decapitated, 4 h after the last dose of drugs, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; <sup>+</sup> $p < 0.05$ , <sup>++</sup> $p < 0.01$  vs. GBR 12,909-treated group; <sup>^</sup> $p < 0.05$ , <sup>^^</sup> $p < 0.01$  vs. GBR 12,909-treated group

and interaction of both treatments ( $F_{1,26} = 4.02$ , *ns*) on NM level in the frontal cortex (Table 2).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,25} = 0.6$ , *ns*), treatment 2 ( $F_{1,25} = 0.01$ , *ns*), and interaction of both treatments ( $F_{1,25} = 1.17$ , *ns*) on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 2).

#### Serotonin (5-HT) and Its Metabolite

Two-way ANOVA revealed an insignificant ( $F_{1,25} = 0.19$ , *ns*) effect of treatment 1 on 5-HT amount in the frontal cortex. The same analysis showed a significant effect of treatment 2 ( $F_{1,25} = 9.56$ ,  $p < 0.01$ ) on 5-HT amount and insignificant effect of interaction of both treatments ( $F_{1,25} = 0.48$ , *ns*). The post hoc test showed a significant increase in 5-HT level after GBR 12909 administration alone and combined with BSO compared to control animals ( $p < 0.05$ ) (Table 2).

Two-way ANOVA indicated an insignificant ( $F_{1,26} = 0.01$ , *ns*) effect of treatment 1 on 5-HIAA amount. The same analysis showed a significant effect of treatment 2 ( $F_{1,26} = 11.63$ ,  $p < 0.01$ ) on 5-HIAA amount and insignificant effect of interaction of both treatments ( $F_{1,26} = 0.37$ , *ns*). The post hoc test showed a significant increase in 5-HIAA level after GBR 12909 administration compared to control animals ( $p < 0.05$ ) (Table 2).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,25} = 0.49$ , *ns*), treatment 2 ( $F_{1,25} = 0.01$ , *ns*), and interaction of both treatments ( $F_{1,25} = 0.01$ , *ns*) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 2).

#### The Effects of Combined Administration of BSO (3.8 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Hippocampus of 16-Day-Old Rats

##### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed an insignificant effect of treatment 1 (BSO 3.8 mmol;  $F_{1,26} = 0.33$ , *ns*), treatment 2 (GBR 12909 5 mg;  $F_{1,26} = 1.43$ , *ns*), and interaction of both treatments ( $F_{1,26} = 0.28$ , *ns*) on hippocampal DA level (Table 3).

The same analysis revealed an insignificant effect of treatment 1 ( $F_{1,27} = 0.88$ , *ns*), treatment 2 ( $F_{1,27} = 1.77$ , *ns*), and interaction of both treatments ( $F_{1,27} = 0.36$ , *ns*) on DOPAC level (Table 3).

A statistical analysis showed an insignificant ( $F_{1,27} = 2.49$ , *ns*) effect of treatment 1 on 3-MT level, while the effect of treatment 2 ( $F_{1,27} = 4.42$ ,  $p < 0.05$ ) was significant. Interaction of both treatments on 3-MT amount in the hippocampus was found to be insignificant ( $F_{1,27} = 0.02$ , *ns*) (Table 3).

**Table 3** The effects of combined administration of BSO (3.8 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the hippocampus of 16-day-old rats

Hippocampus							
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA	
Vehicle	Vehicle	8	56 ± 7	18 ± 5	12 ± 2	45 ± 3	
Vehicle	GBR 5 mg	8	72 ± 11	30 ± 6	24 ± 7	89 ± 17*	
BSO 3.8 mmol	Sól	8	56 ± 7	28 ± 6	21 ± 5	47 ± 7	
BSO 3.8 mmol	GBR 5 mg	7	62 ± 11	33 ± 9	35 ± 9*	63 ± 6	
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA	
Vehicle	Vehicle	8	210 ± 43	17 ± 3	33 ± 1	62 ± 7	
Vehicle	GBR 5 mg	8	147 ± 16	33 ± 6	72 ± 16*	113 ± 26*	
BSO 3.8 mmol	Vehicle	8	188 ± 27	30 ± 7	41 ± 3	62 ± 7	
BSO 3.8 mmol	GBR 5 mg	7	171 ± 40	26 ± 7	39 ± 6 <sup>+</sup>	63 ± 10 <sup>+</sup>	
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT		
Vehicle	Vehicle	8	83 ± 7	10 ± 2	184 ± 14		
Vehicle	GBR 5 mg	8	122 ± 12*	21 ± 2**	154 ± 7		
BSO 3.8 mmol	Vehicle	8	76 ± 11	15 ± 3	149 ± 9		
BSO 3.8 mmol	GBR 5 mg	7	109 ± 14	16 ± 2	158 ± 18		

The results are expressed as the means ± SEM ( $n=7-8$  animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan's post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (3.8 mmol/kg, *sc*, daily) and GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. The 16-day-old rats were decapitated, 4 h after the last dose of drugs, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; <sup>+</sup> $p < 0.05$ , <sup>++</sup> $p < 0.01$  vs. GBR 12,909-treated group; <sup>^</sup> $p < 0.05$ , <sup>^^</sup> $p < 0.01$  vs. GBR-treated group

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,25} = 1.28$ , *ns*) and a significant effect of treatment 2 ( $F_{1,25} = 8.16$ ,  $p < 0.01$ ) on HVA level in the hippocampus. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,25} = 1.76$ , *ns*) on HVA level in the hippocampus (Table 3).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,27} = 0.84$ , *ns*) and a significant effect of treatment 2 ( $F_{1,27} = 10.26$ ,  $p < 0.01$ ) on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 3). The statistical analysis revealed an insignificant effect of interaction of both treatments ( $F_{1,27} = 0.06$ , *ns*). Duncan's post hoc analysis demonstrated that administration of GBR 12909 increased the rate of dopamine metabolism ( $p < 0.05$ ) (Table 3).

#### Noradrenaline (NA) and Its Metabolite

Two-way ANOVA showed an insignificant ( $F_{1,27} = 0.001$ , *ns*) effect of treatment 1 (BSO 3.8 mmol) and treatment 2 (GBR 5 mg;  $F_{1,27} = 1.53$ , *ns*) on NA level in the hippocampus. Similarly, the effect of interaction of both treatments on NA level in the hippocampus was insignificant ( $F_{1,27} = 0.49$ , *ns*) (Table 3).

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,27} = 0.3$ , *ns*), treatment 2 ( $F_{1,27} = 0.9$ , *ns*), and interaction of both treatments ( $F_{1,27} = 2.3$ , *ns*) on NM level in the hippocampus (Table 3).

Two-way ANOVA indicated an insignificant ( $F_{1,27} = 0.01$ , *ns*) effect of treatment 1 on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 3). The same analysis showed a significant effect of treatment 2 ( $F_{1,27} = 5.11$ ,  $p < 0.05$ ) and insignificant effects of interaction of both treatments ( $F_{1,27} = 3.78$ , *ns*). Duncan's post hoc analysis demonstrated that administration of GBR 12909 increased the rate of noradrenaline metabolism ( $p < 0.01$ ).

#### Serotonin (5-HT) and Its Metabolite

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,25} = 1.6$ , *ns*) and treatment 2 ( $F_{1,25} = 3.6$ , *ns*) on 5-HT amount in the hippocampus. The same analysis showed a significant effect of interaction of both treatments ( $F_{1,25} = 4.45$ ,  $p < 0.05$ ). The post hoc test showed a significant increase in 5-HT level after GBR 12909 administration compared to control animals ( $p < 0.05$ ), and this effect was inhibited by combined treatment with BSO (Table 3).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,25} = 2.33$ , *ns*), treatment 2 ( $F_{1,25} = 2.59$ , *ns*), and interaction of both treatments ( $F_{1,25} = 2.35$ , *ns*) on 5-HIAA amount in the hippocampus. The post hoc test showed a significant increase in 5-HIAA level after GBR 12909 administration compared to control animals ( $p < 0.05$ ), and this effect was inhibited by combined treatment with BSO (Table 3).



Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,25}=1.59, ns$ ), treatment 2 ( $F_{1,25}=0.73, ns$ ), and interaction of both treatments ( $F_{1,25}=2.76, ns$ ) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 3).

**The Effects of Combined Administration of BSO (7.6 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Hippocampus of 16-day-Old Rats**

**Dopamine (DA) and Its Metabolites**

Two-way ANOVA showed an insignificant effect of treatment 1 (BSO 7.6 mmol;  $F_{1,28}=0.38, ns$ ), treatment 2 (GBR 12909 5 mg;  $F_{1,28}=1.61, ns$ ), and interaction of both treatments ( $F_{1,28}=0.38, ns$ ) on the hippocampal DA level (Table 4).

The same analysis revealed an insignificant effect of treatment 1 ( $F_{1,28}=0.18, ns$ ) and treatment 2 ( $F_{1,28}=0.66, ns$ ) on DOPAC level. At the same time, interaction of both treatments on DOPAC amount in the hippocampus was significant ( $F_{1,28}=4.30, p < 0.05$ ) (Table 4).

A statistical analysis showed an insignificant effect of treatment 1 ( $F_{1,28}=2.86, ns$ ), treatment 2 ( $F_{1,28}=0.34, ns$ ), and interaction of both treatments ( $F_{1,28}=1.36, ns$ ) on 3-MT amount in the hippocampus (Table 4).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,27}=0.01, ns$ ) and a significant effect of treatment 2 ( $F_{1,27}=8.43, p < 0.01$ ) on HVA level in the hippocampus. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,27}=0.41, ns$ ) on HVA level in the hippocampus. The post hoc test showed a significant increase in HVA level after GBR administration compared to control animals ( $p < 0.05$ ) (Table 4).

Two-way ANOVA indicated an insignificant ( $F_{1,28}=0.14, ns$ ) effect of treatment 1 on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 4). The same analysis showed a significant effect of treatment 2 ( $F_{1,28}=10.12, p < 0.01$ ) and insignificant effects of interaction of both treatments ( $F_{1,28}=0.001, ns$ ). The Duncan’s test revealed a significant increase the rate of dopamine metabolism after administration of GBR 12909 alone and in combination with BSO 7.6 mmol ( $p < 0.05$ ) (Table 4).

**Noradrenaline (NA) and Its Metabolite**

Two-way ANOVA showed an insignificant ( $F_{1,28}=0.45, ns$ ) effect of treatment 1 (BSO 7.6 mmol) and treatment 2 (GBR 12909 5 mg;  $F_{1,28}=1.36, ns$ ) on NA level in the hippocampus. Similarly, the effect of interaction of both treatments on NA level in the hippocampus was insignificant ( $F_{1,28}=1.12, ns$ ) (Table 4).

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,28}=0.36, ns$ ), treatment 2 ( $F_{1,28}=0.53, ns$ ),

**Table 4** The effects of combined administration of BSO (7.6 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the hippocampus of 16-day-old rats

Hippocampus		N	DA	DOPAC	3-MT	HVA
Treatment 1	Treatment 2					
Vehicle	Vehicle	8	56 ± 7	18 ± 5	12 ± 2	45 ± 3
Vehicle	GBR 5 mg	8	72 ± 11	30 ± 6	24 ± 7	89 ± 17*
BSO 7.6 mmol	Vehicle	8	56 ± 8	25 ± 4	32 ± 9	51 ± 8
BSO 7.6 mmol	GBR 5 mg	8	62 ± 7	19 ± 2	28 ± 8	80 ± 15
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA
Vehicle	Vehicle	8	210 ± 43	17 ± 3	33 ± 1	62 ± 7
Vehicle	GBR 5 mg	8	147 ± 16	33 ± 6	72 ± 16*	113 ± 26
BSO 7.6 mmol	Vehicle	8	161 ± 19	25 ± 8	44 ± 6	57 ± 8
BSO 7.6 mmol	GBR 5 mg	8	158 ± 29	18 ± 5	40 ± 9 <sup>+</sup>	53 ± 6
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT	
Vehicle	Vehicle	8	83 ± 7	10 ± 2	184 ± 14	
Vehicle	GBR 5 mg	8	122 ± 12*	21 ± 2	154 ± 7	
BSO 7.6 mmol	Vehicle	8	88 ± 13	14 ± 3	130 ± 7**	
BSO 7.6 mmol	GBR 5 mg	8	127 ± 15*	13 ± 5	152 ± 18	

The results are expressed as the means ± SEM (n=8 animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan’s post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (7.6 mmol/kg, sc, daily) and GBR 12,909 (5 mg/kg, sc, every second day), alone or in combination in 16-day-old rats; 4 h after the last dose of drugs, the rats were decapitated, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; <sup>+</sup> $p < 0.05$ , <sup>++</sup> $p < 0.01$  vs. GBR 12,909-treated group; <sup>^</sup> $p < 0.05$ , <sup>^^</sup> $p < 0.01$  vs. GBR 12,909-treated group

and interaction of both treatments ( $F_{1,28}=3.77$ , *ns*) on NM level in the hippocampus (Table 4).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28}=0.22$ , *ns*), treatment 2 ( $F_{1,28}=1.92$ , *ns*), and interaction of both treatments ( $F_{1,28}=2.72$ , *ns*) on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 4).

### Serotonin (5-HT) and Its Metabolite

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,27}=0.99$ , *ns*) and treatment 2 ( $F_{1,27}=2.90$ , *ns*) on 5-HT amount in the hippocampus. The same analysis showed a significant effect of interaction of both treatments ( $F_{1,27}=4.23$ ,  $p<0.05$ ). The post hoc test showed a significant increase in 5-HT level after GBR 12909 administration compared to control animals ( $p<0.05$ ), and this effect was inhibited by combined treatment with BSO (Table 4).

Two-way ANOVA indicated a significant effect of treatment 1 ( $F_{1,27}=4.67$ ,  $p<0.05$ ), while the effects of treatment 2 ( $F_{1,27}=2.42$ , *ns*) and interaction of both treatments ( $F_{1,27}=3.36$ , *ns*) on 5-HIAA amount in the hippocampus were insignificant. The post hoc test showed a significant increase in 5-HIAA level after GBR 12909 administration compared to control animals ( $p<0.05$ ) (Table 4).

Two-way ANOVA indicated a significant effect of treatment 1 ( $F_{1,27}=4.86$ ,  $p<0.05$ ) on the rate of serotonin

metabolism measured as [5-HIAA]/[5-HT] (Table 4). The same analysis revealed an insignificant effect of treatment 2 ( $F_{1,27}=0.15$ , *ns*) and interaction of both treatments ( $F_{1,27}=4.19$ , *ns*) on serotonin metabolism (Table 4). The Duncan's test revealed a significant reduction of serotonin metabolism after administration of BSO 7.6 mmol (Table 4).

### The Effects of Combined Administration of BSO (3.8 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Frontal Cortex of 91-Day-Old Rats

#### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed an insignificant ( $F_{1,25}=0.62$ , *ns*) effect of treatment 1 (BSO 3.8 mmol), treatment 2 (GBR 12909 5 mg;  $F_{1,25}=2.87$ , *ns*), and interaction of both treatments ( $F_{1,25}=2.06$ , *ns*) on the frontal DA level (Table 5).

Two-way ANOVA revealed an insignificant ( $F_{1,28}=2.09$ , *ns*) effect of treatment 1 and treatment 2 ( $F_{1,28}=0.005$ , *ns*) on DOPAC level. The effect of interaction of both treatments also was found to be insignificant ( $F_{1,28}=3.01$ , *ns*) (Table 5).

A statistical analysis showed an insignificant ( $F_{1,28}=0.07$ , *ns*) effect of treatment 1 and treatment 2 ( $F_{1,28}=0.01$ , *ns*) on 3-MT level. Interaction of both treatments was found to be insignificant ( $F_{1,28}=0.46$ , *ns*) (Table 5).

**Table 5** The effects of combined administration of BSO (3.8 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the frontal cortex of 91-day-old rats

Frontal cortex						
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA
Vehicle	Vehicle	8	813 ± 102	122 ± 14	28 ± 4	187 ± 18
Vehicle	GBR 5 mg	8	543 ± 85*	101 ± 9	26 ± 4	183 ± 10
BSO 3.8 mmol	Vehicle	8	758 ± 61	119 ± 5	25 ± 3	217 ± 13
BSO 3.8 mmol	GBR 5 mg	8	735 ± 98	141 ± 18 <sup>+</sup>	27 ± 2	234 ± 18* <sup>+</sup>
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA
Vehicle	Vehicle	8	376 ± 12	20 ± 3	221 ± 11	159 ± 7
Vehicle	GBR 5 mg	8	372 ± 6	45 ± 4**	222 ± 7	151 ± 9
BSO 3.8 mmol	Vehicle	8	418 ± 16*	33 ± 5	198 ± 9	133 ± 4*
BSO 3.8 mmol	GBR 5 mg	8	402 ± 11	42 ± 7**	227 ± 12	156 ± 6 <sup>^</sup>
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT	
Vehicle	Vehicle	8	22 ± 2	5 ± 1	73 ± 5	
Vehicle	GBR 5 mg	8	39 ± 5**	12 ± 1**	68 ± 4	
BSO 3.8 mmol	Vehicle	8	30 ± 2	8 ± 2	68 ± 2	
BSO 3.8 mmol	GBR 5 mg	8	32 ± 3	10 ± 2*	70 ± 4	

The results are expressed as the means ± SEM ( $n=7-8$  animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan's post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (3.8 mmol/kg, *sc.*, daily) and GBR 12,909 (5 mg/kg, *sc.*, every second day), alone or in combination. Control pups were given vehicle instead of the BSO and GBR 12,909. The 91-day-old rats were decapitated, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p<0.05$ , \*\* $p<0.01$  vs. vehicle-treated group; <sup>+</sup> $p<0.05$ , <sup>++</sup> $p<0.01$  vs. GBR 12,909-treated group; <sup>^</sup> $p<0.05$ , <sup>^^</sup> $p<0.01$  vs. GBR 12,909-treated group

Two-way ANOVA indicated a significant effect of treatment 1 ( $F_{1,28} = 6.95$ ,  $p < 0.01$ ) on HVA level in the frontal cortex. The same analysis showed an insignificant effect of treatment 2 ( $F_{1,28} = 0.16$ ,  $ns$ ) and interaction of both treatments ( $F_{1,28} = 0.45$ ,  $ns$ ) on HVA level in the frontal cortex (Table 5). The post hoc test indicated elevation of HVA level after combined treatment with BSO and GBR 12909 ( $p < 0.05$ ) (Table 5).

Two-way ANOVA indicated an insignificant ( $F_{1,28} = 0.05$ ,  $ns$ ) effect of treatment 1 on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 5). The same analysis showed a significant effect of treatment 2 ( $F_{1,28} = 7.58$ ,  $p < 0.05$ ) and insignificant effects of interaction of both treatments ( $F_{1,28} = 3.94$ ,  $ns$ ). The Duncan's test revealed a significant increase the rate of dopamine metabolism after administration of GBR 12909 ( $p < 0.01$ ) (Table 5).

### Noradrenaline (NA) and Its Metabolite

Two-way ANOVA showed a significant ( $F_{1,28} = 9.04$ ,  $p < 0.01$ ) effect of treatment 1 (BSO 3.8 mmol) on the frontal NA level. At the same time, the effect of treatment 2 (GBR 5 mg;  $F_{1,28} = 0.67$ ,  $ns$ ) and interaction of both treatments on NA level in the frontal cortex were insignificant ( $F_{1,28} = 0.22$ ,  $ns$ ). The Duncan's test revealed increased NA amount after BSO administration compared to saline ( $p < 0.05$ ) (Table 5).

Two-way ANOVA revealed an insignificant ( $F_{1,28} = 0.90$ ,  $ns$ ) effect of treatment 1 on NM level. The same analysis showed a significant effect of treatment 2 ( $F_{1,28} = 1.99$ ,  $p < 0.01$ ) on NM amount and insignificant effect of interaction of both treatments ( $F_{1,28} = 2.51$ ,  $ns$ ). The post hoc test revealed significantly increased level of NM in the GBR 12909 group when compared to saline ( $p < 0.01$ ). Similar effect was observed in the combined treatment group (BSO + GBR 12909;  $p < 0.01$ ) (Table 5).

Two-way ANOVA indicated an insignificant ( $F_{1,28} = 0.14$ ,  $ns$ ) effect of treatment 1 on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 5). The same analysis showed a significant effect of treatment 2 ( $F_{1,28} = 11.41$ ,  $p < 0.01$ ) and insignificant effects of interaction of both treatments ( $F_{1,28} = 2.53$ ,  $ns$ ). The Duncan's test revealed a significant elevation the rate of noradrenaline metabolism after administration of GBR 12909 alone and in combination with BSO ( $p < 0.01$  and  $p < 0.05$ ), respectively (Table 5).

### Serotonin (5-HT) and Its Metabolite

Two-way ANOVA revealed an insignificant ( $F_{1,28} = 0.84$ ,  $ns$ ) effect of treatment 1, treatment 2 ( $F_{1,28} = 1.99$ ,  $ns$ ), and

interaction of both treatments ( $F_{1,28} = 1.89$ ,  $ns$ ) on 5-HT amount in the frontal cortex (Table 5).

Two-way ANOVA indicated an insignificant ( $F_{1,28} = 2.03$ ,  $ns$ ) effect of treatment 1 and treatment 2 ( $F_{1,28} = 1.14$ ,  $ns$ ) on 5-HIAA amount. The same analysis showed a significant effect of interaction of both treatments ( $F_{1,28} = 4.54$ ,  $p < 0.05$ ) on 5-HIAA amount. The post hoc test showed a significant decrease in 5-HIAA level after BSO administration compared to control animals ( $p < 0.05$ ) (Table 5).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 0.16$ ,  $ns$ ), treatment 2 ( $F_{1,28} = 0.12$ ,  $ns$ ), and interaction of both treatments ( $F_{1,28} = 0.8$ ,  $ns$ ) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 5).

## The Effects of Combined Administration of BSO (7.6 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Frontal Cortex of 91-Day-Old Rats

### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed an insignificant ( $F_{1,24} = 0.68$ ,  $ns$ ) effect of treatment 1 (BSO 3.8 mmol), treatment 2 (GBR 12909 5 mg;  $F_{1,24} = 3.18$ ,  $ns$ ), and interaction of both treatments ( $F_{1,24} = 1.88$ ,  $ns$ ) on the frontal DA level (Table 6).

Two-way ANOVA revealed an insignificant ( $F_{1,28} = 4.17$ ,  $ns$ ) effect of treatment 1 and treatment 2 ( $F_{1,28} = 1.41$ ,  $ns$ ) on DOPAC level. Similarly, the effect of interaction of both treatments was also insignificant ( $F_{1,28} = 0.66$ ,  $ns$ ). The Duncan's test revealed that combined treatment with both compounds (BSO + GBR 12909) decreased DOPAC level in comparison with the BSO group ( $p < 0.05$ ) (Table 6).

A statistical analysis showed an insignificant ( $F_{1,28} = 1.27$ ,  $p = ns$ ) effect of treatment 1 and treatment 2 ( $F_{1,28} = 1.01$ ,  $ns$ ), but interaction of both treatments on 3-MT level in the frontal cortex was significant ( $F_{1,28} = 0.27$ ,  $ns$ ) (Table 6).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 0.79$ ,  $ns$ ) and treatment 2 ( $F_{1,28} = 0.81$ ,  $ns$ ) on HVA level in the frontal cortex. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,28} = 1.09$ ,  $ns$ ) on HVA level in the frontal cortex (Table 6).

Two-way ANOVA indicated an insignificant ( $F_{1,28} = 0.57$ ,  $ns$ ) effect of treatment 1 on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 6). The same analysis showed a significant effect of treatment 2 ( $F_{1,28} = 5.83$ ,  $p < 0.05$ ) and insignificant effects of interaction of both treatments ( $F_{1,28} = 1.66$ ,  $ns$ ). The Duncan's test revealed a significant increase the rate of dopamine metabolism after administration of GBR 12909 alone and in combination with BSO ( $p < 0.05$ ) (Table 6).

**Table 6** The effects of combined administration of BSO (7.6 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the frontal cortex of 91-day-old rats

Fontal cortex							
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA	
Vehicle	Vehicle	8	813 ± 102	122 ± 14	28 ± 4	187 ± 18	
Vehicle	GBR 5 mg	8	543 ± 85*	101 ± 9	26 ± 4	183 ± 10	
BSO 7.6 mmol	Vehicle	8	625 ± 73	93 ± 9	25 ± 4	183 ± 6	
BSO 7.6 mmol	GBR 5 mg	8	590 ± 81	89 ± 9*	19 ± 4	238 ± 53	
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA	
Vehicle	Vehicle	8	376 ± 12	20 ± 3	221 ± 11	159 ± 7	
Vehicle	GBR 5 mg	8	372 ± 6	45 ± 4**	222 ± 7	151 ± 9	
BSO 7.6 mmol	Vehicle	8	422 ± 21	34 ± 5*	246 ± 8	160 ± 5	
BSO 7.6 mmol	GBR 5 mg	8	382 ± 19	42 ± 3**	214 ± 9 <sup>^</sup>	142 ± 8	
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT		
Vehicle	Vehicle	8	22 ± 2	5 ± 1	73 ± 5		
Vehicle	GBR 5 mg	8	39 ± 5*	12 ± 1**	68 ± 4		
BSO 7.6 mmol	Vehicle	8	31 ± 3	8 ± 1	65 ± 2		
BSO 7.6 mmol	GBR 5 mg	8	36 ± 6*	11 ± 1**	67 ± 4		

The results are expressed as the means ± SEM ( $n=7-8$  animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan's post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (7.6 mmol/kg, *sc*, daily) and GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. The 91-day-old rats were decapitated, and the tissue (hippocampus, frontal cortex and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; + $p < 0.05$ , ++ $p < 0.01$  vs. GBR 12,909-treated group; <sup>^</sup> $p < 0.05$ , <sup>^^</sup> $p < 0.01$  vs. GBR 12,909-treated group

### Noradrenaline (NA) and Its Metabolite

Two-way ANOVA showed an insignificant ( $F_{1,28} = 3.00$ , *ns*) effect of treatment 1 (BSO 7.6 mmol) and treatment 2 (GBR 12909 5 mg;  $F_{1,28} = 1.98$ , *ns*) on NA level. Similarly, the effect of interaction of both treatments on NA level in the frontal cortex was insignificant ( $F_{1,28} = 1.31$ , *ns*) (Table 6).

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,27} = 1.62$ , *ns*). The same analysis indicated a significant effect of treatment 2 ( $F_{1,27} = 17.62$ ,  $p < 0.01$ ) and interaction of both treatments ( $F_{1,27} = 4.71$ ,  $p < 0.05$ ) on NM level in the frontal cortex (Table 6). The post hoc test revealed significantly increased level of NM in both BSO ( $p < 0.05$ ) and GBR 12909 ( $p < 0.01$ ) group when compared to saline. Similar effect was observed in the combined treatment group (BSO + GBR 12909;  $p < 0.01$ ) (Table 6).

Two-way ANOVA indicated an insignificant ( $F_{1,28} = 0.43$ , *ns*) effect of treatment 1 on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 6). The same analysis showed a significant effect of treatment 2 ( $F_{1,28} = 26.73$ ,  $p < 0.01$ ) and insignificant effects of interaction of both treatments ( $F_{1,28} = 3.84$ , *ns*). The Duncan's test revealed a significant increase the rate of noradrenaline metabolism after administration of GBR 12909 alone and in combination with BSO ( $p < 0.01$ ) (Table 6).

### Serotonin (5-HT) and Its Metabolite

A statistical analysis showed an insignificant ( $F_{1,28} = 0.95$ , *ns*) effect of treatment 1 and treatment 2 ( $F_{1,28} = 3.01$ , *ns*), but interaction of both treatments on 5-HT level in the frontal cortex was significant ( $F_{1,28} = 3.36$ , *ns*) (Table 6).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 0.24$ , *ns*) and treatment 2 ( $F_{1,28} = 2.87$ , *ns*) on 5-HIAA level in the frontal cortex. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,28} = 0.50$ , *ns*) on 5-HIAA level in the frontal cortex (Table 6).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 1.41$ , *ns*), treatment 2 ( $F_{1,28} = 0.21$ , *ns*), and interaction of both treatments ( $F_{1,28} = 0.65$ , *ns*) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 6).

### The Effects of Combined Administration of BSO (3.8 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Hippocampus of 91-Day-Old Rats

#### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed a significant effect of treatment 1 (BSO 3.8 mmol;  $F_{1,28} = 9.50$ ,  $p < 0.01$ ) on the hippocampal DA level. At the same time, the effects of treatment 2 (GBR

12909 5 mg;  $F_{1,28}=2.25, ns$ ) and interaction of both treatments ( $F_{1,28}=1.64, ns$ ) on DA amount were insignificant (Table 7). The Duncan’s test revealed a significant elevation of DA amount after administration of both GBR 12909 or BSO alone and in combination ( $p < 0.05, p < 0.01, \text{ and } p < 0.01$ , respectively) (Table 7).

The same analysis revealed an insignificant effect of treatment 1 ( $F_{1,28}=0.92, ns$ ), treatment 2 ( $F_{1,28}=0.13, ns$ ), and interaction of both treatments ( $F_{1,28}=1.84, ns$ ) on DOPAC level (Table 7).

A statistical analysis showed an insignificant effect of treatment 1 ( $F_{1,28}=1.14, ns$ ), treatment 2 ( $F_{1,28}=0.20, ns$ ), and interaction of both treatments ( $F_{1,28}=1.83, ns$ ) on 3-MT amount (Table 7).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28}=0.05, ns$ ) and treatment 2 ( $F_{1,28}=1.46, ns$ ) on HVA level in the hippocampus. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,28}=0.03, ns$ ) on HVA level in the hippocampus (Table 7).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28}=1.53, ns$ ), treatment 2 ( $F_{1,28}=3.93, ns$ ), and interaction of both treatments ( $F_{1,28}=1.54, ns$ ) on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 7).

**Noradrenaline (NA) and Its Metabolite**

Two-way ANOVA showed a significant ( $F_{1,28}=7.00, p < 0.01$ ) effect of treatment 1 (BSO 3.8 mmol) on NA level in the hippocampus. In contrast, the effects of treatment 2

(GBR 12909 5 mg;  $F_{1,28}=0.33, ns$ ) and interaction of both treatments on NA level in the hippocampus were insignificant ( $F_{1,28}=1.20, ns$ ) (Table 7).

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,26}=0.52, ns$ ), treatment 2 ( $F_{1,26}=2.59, ns$ ), and interaction of both treatments ( $F_{1,26}=1.48, ns$ ) on NM level in the hippocampus (Table 7).

Two-way ANOVA indicated an insignificant ( $F_{1,28}=0.01, ns$ ) effect of treatment 1 on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 7). The same analysis showed a significant effect of treatment 2 ( $F_{1,28}=5.0, p < 0.05$ ) and insignificant effects of interaction of both treatments ( $F_{1,28}=3.92, ns$ ). The Duncan’s test revealed a significant increase the rate of noradrenaline metabolism after administration of GBR 12909 ( $p < 0.01$ ) (Table 7).

**Serotonin (5-HT) and Its Metabolite**

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,28}=0.65, ns$ ), treatment 2 ( $F_{1,28}=0.63, ns$ ), and interaction of both treatments ( $F_{1,28}=0.02, ns$ ) on 5-HT amount in the hippocampus (Table 7).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28}=2.19, ns$ ) on 5-HIAA amount in the hippocampus, while the effect of treatment 2 ( $F_{1,28}=5.01, p < 0.05$ ) was significant. The same analysis showed that interaction of both treatments on the 5-HIAA level ( $F_{1,28}=0.10, ns$ ) was insignificant. The post hoc test showed

**Table 7** The effects of combined administration of BSO (3.8 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the hippocampus of 91-day-old rats

Hippocampus						
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA
Vehicle	Vehicle	8	29 ± 1	12 ± 2	12 ± 2	27 ± 4
Vehicle	GBR 5 mg	8	35 ± 2*	15 ± 1	16 ± 3	22 ± 3
BSO 3.8 mmol	Vehicle	8	39 ± 3**	16 ± 2	18 ± 2	27 ± 3
BSO 3.8 mmol	GBR 5 mg	8	39 ± 3**	14 ± 1	16 ± 3	24 ± 2
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA
Vehicle	Vehicle	8	401 ± 8	16 ± 2	129 ± 10	120 ± 4
Vehicle	GBR 5 mg	8	378 ± 10	23 ± 2	140 ± 12	135 ± 7
BSO 3.8 mmol	Vehicle	8	422 ± 20	21 ± 2	140 ± 12	129 ± 5
BSO 3.8 mmol	GBR 5 mg	8	429 ± 13 <sup>+</sup>	22 ± 3	147 ± 12	148 ± 12*
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT	
Vehicle	Vehicle	8	94 ± 14	4 ± 0.4	96 ± 7	
Vehicle	GBR 5 mg	8	62 ± 8*	6 ± 0.5**	99 ± 6	
BSO 3.8 mmol	Vehicle	8	69 ± 9	5 ± 0.4	96 ± 8	
BSO 3.8 mmol	GBR 5 mg	8	62 ± 7*	5 ± 0.6	103 ± 9	

The results are expressed as the means ± SEM ( $n=7-8$  animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan’s post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (3.8 mmol/kg, *sc*, daily) and GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. The 91-day-old rats were decapitated, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; <sup>+</sup> $p < 0.05$ , <sup>++</sup> $p < 0.01$  vs. GBR 12,909-treated group; <sup>^</sup> $p < 0.05$ , <sup>^^</sup> $p < 0.01$  vs. GBR-treated group



a significant increase in 5-HIAA level after combined treatment with GBR 12909 and BSO compared to control animals ( $p < 0.05$ ) (Table 7).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 0.06$ , *ns*), treatment 2 ( $F_{1,28} = 0.44$ , *ns*), and interaction of both treatments ( $F_{1,28} = 0.06$ , *ns*) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 7).

### The Effects of Combined Administration of BSO (7.6 mmol/kg) and GBR 12909 (5 mg/kg) on the Monoamine Metabolism in the Hippocampus of 91-Day-Old Rats

#### Dopamine (DA) and Its Metabolites

Two-way ANOVA showed a significant effect of treatment 1 (BSO 7.6 mmol;  $F_{1,28} = 12.78$ ,  $p < 0.01$ ) and treatment 2 (GBR 12909 5 mg;  $F_{1,28} = 9.20$ ,  $p < 0.01$ ) on the hippocampal DA level. At the same time, the interaction of both treatments ( $F_{1,28} = 0.07$ , *ns*) was insignificant (Table 8). The Duncan's test revealed a significant elevation of DA amount after administration of BSO alone and in combination with GBR 12909 ( $p < 0.05$  and  $p < 0.01$ , respectively) (Table 8).

The same analysis revealed a significant effect of treatment 1 ( $F_{1,28} = 8.15$ ,  $p < 0.01$ ) on DOPAC level. At the same time, the effects of treatment 2 ( $F_{1,28} = 1.50$ , *ns*) and interaction of both treatments on DOPAC amount in the

hippocampus were insignificant ( $F_{1,28} = 0.29$ , *ns*) (Table 8). The post hoc test indicated a significant elevation of DOPAC amount after administration of BSO alone and in combination with GBR 12909 ( $p < 0.05$ ) (Table 8).

A statistical analysis showed an insignificant effect of treatment 1 ( $F_{1,28} = 1.51$ , *ns*), treatment 2 ( $F_{1,28} = 0.68$ , *ns*), and interaction of both treatments ( $F_{1,28} = 0.76$ , *ns*) on 3-MT amount in the hippocampus (Table 8).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 2.15$ , *ns*) and treatment 2 ( $F_{1,28} = 0.04$ , *ns*) on HVA level in the hippocampus. The same analysis showed an insignificant effect of interaction of both treatments ( $F_{1,28} = 1.03$ , *ns*) on HVA level in the hippocampus (Table 8).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28} = 0.01$ , *ns*), treatment 2 ( $F_{1,28} = 2.64$ , *ns*), and interaction of both treatments ( $F_{1,28} = 1.24$ , *ns*) on the rate of dopamine metabolism measured as [HVA]/[DA] (Table 8).

#### Noradrenaline (NA) and Its Metabolite

Two-way ANOVA showed a significant ( $F_{1,28} = 2.82$ ,  $p < 0.01$ ) effect of treatment 1 (BSO 7.6 mmol) and treatment 2 (GBR 12909 5 mg;  $F_{1,28} = 12.69$ ,  $p < 0.01$ ) on NA level in the hippocampus. At the same time, the effect of interaction of both treatments on NA level in the hippocampus was

**Table 8** The effects of combined administration of BSO (7.6 mmol/kg) and GBR 12,909 (5 mg/kg) on the monoamine metabolism in the hippocampus of 91-day-old rats

Hippocampus						
Treatment 1	Treatment 2	N	DA	DOPAC	3-MT	HVA
Vehicle	Vehicle	8	29 ± 1	12 ± 2	12 ± 2	27 ± 4
Vehicle	GBR 5 mg	8	35 ± 2	15 ± 1	16 ± 3	22 ± 3
BSO 7.6 mmol	Vehicle	8	37 ± 2*	18 ± 2*	17 ± 3	29 ± 5
BSO 7.6 mmol	GBR 5 mg	8	44 ± 3*** <sup>^</sup>	19 ± 2 *	17 ± 2	32 ± 3
Treatment 1	Treatment 2		NA	NM	5-HT	5-HIAA
Vehicle	Vehicle	8	401 ± 8	16 ± 2	129 ± 10	120 ± 4
Vehicle	GBR 5 mg	8	378 ± 10	23 ± 2	140 ± 12	135 ± 7
BSO 7.6 mmol	Vehicle	8	502 ± 16**	31 ± 5*	145 ± 8	142 ± 8*
BSO 7.6 mmol	GBR 5 mg	8	438 ± 13*** <sup>^</sup>	30 ± 4*	138 ± 8	135 ± 6
Treatment 1	Treatment 2		HVA/DA	NM/NA	5-HIAA/5-HT	
Vehicle	Vehicle	8	94 ± 14	4 ± 0.4	96 ± 7	
Vehicle	GBR 5 mg	8	62 ± 8	6 ± 0.5	99 ± 6	
BSO 7.6 mmol	Vehicle	8	80 ± 15	6 ± 1	100 ± 8	
BSO 7.6 mmol	GBR 5 mg	8	74 ± 7	7 ± 0.7*	100 ± 6	

The results are expressed as the means ± SEM ( $n = 8$  animals per group). Data were analyzed with a two-way ANOVA followed by the Duncan's post hoc test. Between the postnatal days p5 and p16, male Sprague–Dawley pups were treated with BSO (7.6 mmol/kg, *sc*, daily) and GBR 12,909 (5 mg/kg, *sc*, every second day), alone or in combination. The 91-day-old rats were decapitated, and the tissue (hippocampus, frontal cortex, and striatum) for biochemical assays was dissected. Statistical significance: \* $p < 0.05$ , \*\* $p < 0.01$  vs. vehicle-treated group; + $p < 0.05$ , ++ $p < 0.01$  vs. GBR 12,909-treated group; ^ $p < 0.05$ , ^^ $p < 0.01$  vs. GBR 12,909-treated group

insignificant ( $F_{1,28}=2.87$ , *ns*) (Table 8). The post hoc test indicated a significant elevation of NA amount after administration of BSO alone and in combination with GBR 12909 ( $p < 0.01$  and  $p < 0.05$ , respectively) (Table 8).

Two-way ANOVA revealed a significant effect of treatment 1 ( $F_{1,25}=7.78$ ,  $p < 0.01$ ), while the effects of treatment 2 ( $F_{1,25}=0.68$ , *ns*) and interaction of both treatments ( $F_{1,25}=1.04$ , *ns*) on NM level in the hippocampus were insignificant (Table 8). The Duncan's test indicated a significant elevation of NM amount after administration of BSO alone and in combination with GBR 12909 ( $p < 0.05$ ) (Table 8).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28}=3.26$ , *ns*), treatment 2 ( $F_{1,28}=2.62$ , *ns*), and interaction of both treatments ( $F_{1,28}=1.2$ , *ns*) on the rate of noradrenaline metabolism measured as [NM]/[NA] (Table 8).

### Serotonin (5-HT) and Its Metabolite

Two-way ANOVA revealed an insignificant effect of treatment 1 ( $F_{1,28}=0.58$ , *ns*), treatment 2 ( $F_{1,28}=0.04$ , *ns*), and interaction of both treatments ( $F_{1,28}=0.86$ , *ns*) on 5-HT amount in the hippocampus (Table 8).

The statistical analysis indicated an insignificant effect of treatment 1 ( $F_{1,28}=3.18$ , *ns*), treatment 2 ( $F_{1,28}=0.37$ , *ns*), and interaction of both treatments ( $F_{1,28}=2.85$ , *ns*) on 5-HIAA amount in the hippocampus (Table 8).

Two-way ANOVA indicated an insignificant effect of treatment 1 ( $F_{1,28}=0.1$ , *ns*), treatment 2 ( $F_{1,28}=0.04$ , *ns*), and interaction of both treatments ( $F_{1,28}=0.05$ , *ns*) on the rate of serotonin metabolism measured as [5-HIAA]/[5-HT] (Table 8).

## Discussion

It is known that disturbed sensorimotor gating is a multimodal and cross-species phenomenon observed in schizophrenia (Moriwaki et al. 2009; Mena et al. 2016). In the present study, we evaluated the impact of repeated treatment with BSO in two doses (3.8 and 7.6 mmol/kg) alone and in combination with GBR 12909 on prepulse inhibition of the acoustic startle response (PPI) in adult (p90) male rats. Although the analysis did not reveal statistically relevant changes, we noticed that the administration of BSO only at a dose of 3.8 mmol/kg resulted in a tendency to decrease PPI for all tested prepulse intensities. In contrast, we did not find a similar effect for the animals exposed to BSO at 7.6 mmol/kg and also after combined treatment with BSO and GBR 12909. In another experiment, the authors also did not demonstrate a decrease in PPI for all tested prepulse intensities (Preissmann et al. 2016).

In our previous behavioral study (Górny et al. 2019), we demonstrated that inhibition of glutathione synthesis by repeated administration of BSO alone or in combination with GBR 12909 to Sprague–Dawley pups in early postnatal life (p5–p16) induced the schizophrenia-like behavior evaluated in the social interaction test and in the novel object recognition test in early adulthood (p90). Moreover, we also checked their impact on the levels of glutathione and sulfur amino acids (cysteine, methionine) and on the global DNA methylation in the prefrontal cortex and hippocampus (Górny et al. 2019). These data suggest that transient alterations in the content of glutathione and sulfur amino acid methionine during early postnatal life lead to changes in epigenetic status in the prefrontal cortex and hippocampus and to manifestation of social and cognitive deficits in adult rats. To further characterize this rat model of schizophrenia, in a recently published study (Górny et al. 2020), the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione disulfide reductase) and the levels of lipid peroxidation were analyzed in relation to glutathione content and sulfur amino acids, methionine, and cysteine in the prefrontal cortex and hippocampus of 16-day-old rats from the group treated with BSO + GBR 12909 (Górny et al. 2020). This analysis showed that chronic administration of the BSO + GBR 12909 combination resulted in a significant reduction in the level of lipid peroxidation in the examined brain structures, indicating a weakening of the oxidative power of their cells and ultimately leading to changes in redox cell signaling. As a result of the redox state disturbance in the examined brain structures in the early postnatal life, social and cognitive deficits may occur in adulthood.

Continuing research on the molecular mechanism of action of BSO and GBR 12909, we evaluated the influence of chronic treatment with both compounds on the monoamine levels in two main brain structures involved in the regulation of working memory (frontal cortex and the hippocampus). As shown in Tables 1 and 3, a low dose of BSO (3.8 mmol) did not cause changes in the levels and metabolism of monoamines in both the frontal cortex and the hippocampus in 16-day-old rats. At the same time, the higher dose of BSO (7.6 mmol) induced an increase in DA (30%) and 3-MT (50%) levels in the frontal cortex in 16-day-old rats (Table 2). On the other hand, GBR 12909 induced a significant increase in the level of DA (100%) and its metabolites, 3-MT (40%) and HVA (30%), as well as an increase in the levels of 5-HT (50%) and 5-HIAA (50%) in the frontal cortex of 16-day-old rats (Tables 1 and 2). On the other hand in the hippocampus, treatment with GBR induced only an increase in HVA (50%), 5-HT (110%), and 5-HIAA (90%) (Tables 3 and 4). There are evidences that overactivity of the dopaminergic system may produce some symptoms of schizophrenia (Stone et al. 2007). Our

current results indicated an increase in the rate of total DA metabolism measured by the [HVA]/[DA] index after both GBR 12909 alone treatment and co-administration with BSO (Tables 1, 2, 3, and 4). Similarly, treatment with GBR 12909 alone and together with BSO increased the rate of total NA metabolism as measured by the [NM]/[NA] index (Tables 1, 2, 3, and 4). In rodent models, DA is involved in the regulation of sensorimotor gating (PPI) (see a review by Ott and Nieder 2019; Swerdlow et al. 1991). Geyer et al. (2001) showed that dopamine D<sub>2</sub> receptor played a key role in mediating the effect of dopamine on PPI. Both typical and atypical antipsychotics inhibit disruption of PPI induced by the dopamine agonist, apomorphine (Geyer et al. 2001). Their ability to reverse these deficits was related to their affinity and efficacy for dopamine D<sub>2</sub> receptor (Swerdlow et al. 1994a, b, 2001). On the other hand, in schizophrenic patients, PPI deficit is attenuated only by some atypical neuroleptics (e.g., clozapine and olanzapine) (Duncan et al. 2003; Wynn et al. 2007). Our present results demonstrated that combined administration of BSO and GBR 12909 restored DA contents to control levels while leaving high levels of HVA (30%) in the frontal cortex of 16-day-old rats (Tables 1 and 2). In this group, we also observed a decrease in the level of NA (15%) and a simultaneous increase in the level of its metabolite, NM (50%) (Table 1). On the other hand, in the hippocampus, the combined administration of BSO and GBR 12909 restored the levels of 5-HT and 5-HIAA to the control level (Table 3). Serotonin acts as a modulator of anxiety, and the increase in serotonin concentration produces anxiolytic effect (Graeff et al. 1996).

The results of *ex vivo* studies showed that in the frontal cortex of 91-day-old rats, BSO (3.8 mmol) increased the level of NA (15%) and simultaneously decreased the level of 5-HIAA (20%) (Table 5), while in the hippocampus, it significantly increased the level of DA (30%) (Table 7). The higher dose of BSO (7.6 mmol) increased the NM level (60%) in the frontal cortex of 91-day-old rats, while in the hippocampus, it caused a significant increase in the level of DA (15%) and its metabolite DOPAC (30%) and NA (30%) and its metabolite, NM (50%) (Table 8). Moreover, GBR significantly reduced DA level (30%) in the frontal cortex and simultaneously elevated DA concentration (10%) in the hippocampus in 91-day-old rats (Tables 5, 6, and 7). Our current results indicated that in the frontal cortex of 91-day-old rats, both GBR 12909 given alone and combined with BSO increased the total rate of DA and NA metabolism, while in the hippocampus, both of these indices were lowered (Tables 5, 6, 7, and 8). The results from psychological, pharmacological, and biochemical studies indicated that both positive and negative symptoms of schizophrenia might be related of the dysregulation of the noradrenergic system, e.g., elevated NA concentration was found in both the blood

plasma and cerebrospinal fluid of patients with schizophrenia (see review Mäki-Marttunen et al. 2020; Savransky et al. 2021).

Pre-clinical and clinical studies demonstrated that both DA and NA played important roles in working memory. Interestingly, both excessive and decreased concentrations of DA and NA can induce deficits in working memory and cognitive impairment (Arnsten 1997; Białoń et al. 2020; Inagaki et al. 2010; Pietraszek et al. 2009; Wąsik et al. 2019). The current results obtained with the frontal cortex of 90-day-old rats indicate that the combined injections of BSO and GBR 12909 maintain monoamine concentrations at physiological levels (Tables 5 and 6). On the other hand, in the hippocampus, we observed a weak increase in DA (15%) and NA (10%) levels in the combined BSO + GBR 12909 groups (Tables 7 and 8).

In summary, the treatment with BSO or GBR 12909 alone induces weak changes in the activity of dopaminergic, serotonergic, and noradrenergic neurons in the two major brain structures responsible for working memory. As a result, the PPI abnormalities observed in the behavioral test are weak and non-significant. However, the combined administration of both substances allows for maintaining the normal activity of monoaminergic systems in the rat brain.

The most important changes in the functioning of monoaminergic systems were observed in the frontal cortex of 16-day-old rats. Therefore, it seems that the frontal cortex of rat puppies is most sensitive to glutathione deficiencies resulting in increased oxidative stress in neurons. As a result, it can lead to cognitive and memory impairment.

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## Declarations

**Conflict of Interest** The authors declare no competing interests.

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