Neuronal correlates underlying the role of the zinc sensing receptor (GPR39) in passive-coping behaviour

Anupam Sah, Maria Kharitonova, Katarzyna Mlyniec

Department of Pharmacology, Medical University of Innsbruck, Innsbruck, Austria
Department of Pharmacology and Toxicology, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 80-82/III, A-6020, Innsbruck, Austria
Department of Pharmacobiology, Jagiellonian University Medical College, Medyczna 9, PL 30-688, Krakow, Poland

ARTICLE INFO

Keywords: GPR39 Antidepressants Zinc Depression c-Fos

ABSTRACT

The Zn\textsuperscript{2+} receptor GPR39 is proposed to be involved in the pathophysiology of depression. GPR39 knockout (KO) animals show depressive- and anxiety-like behaviour, and resistance to conventional monoamine-based antidepressants. However, it is unclear as to which brain regions are involved in the pro-depressive phenotype of GPR39KO mice and the resistance to monoamine-targeting antidepressant treatment. Our current study confirmed previous results, showing that mice lacking GPR39 display enhanced passive coping-like behaviour compared with their wild-type controls. Furthermore, this study shows for the first time that GPR39KO displayed aberrant challenge-induced neuronal activity in key brain regions associated with passive coping behaviour. Imipramine induced only a marginal reduction in the enhanced passive coping behaviour in GPR39KO mice, which was associated with attenuation of the hyperactive prefrontal cortex. Furthermore, this study shows for the first time that GPR39KO displayed aberrant challenge-induced neuronal activity in key brain regions associated with passive coping behaviour. Imipramine induced only a marginal reduction in the enhanced passive coping behaviour in GPR39KO mice, which was associated with attenuation of the hyperactive prefrontal cortex. Similarly, the aberrant activity within the amygdalar subregions was normalized following imipramine treatment in the GPR39KO mice, indicating that imipramine mediates these effects independently of GPR39 in the prefrontal cortex and amygdala. However, imipramine failed to modulate the aberrant brain activity in other brain regions, such as the anterior CA3 and the dentate gyrus, in GPR39KO mice. Normalization of aberrant activity in these areas has been shown previously to accompany successful behavioural effects of antidepressants. Taken together, our data suggest that monoamine-based antidepressants such as imipramine exert their action via GPR39-dependent and -independent pathways. Failure to modulate passive-coping related aberrant activity in important brain areas of the depression circuitry is proposed to mediate/contribute to the greatly reduced antidepressant action of monoamine-based antidepressants in GPR39KO mice.

1. Introduction

Zinc is one of the most important trace elements that play a significant role in mood disorders (Swardfager et al., 2013; Mlyniec et al., 2014b). Lower serum zinc was found in depressed patients and it has been proposed as a state marker of that illness (Maes et al., 1994; Siwek et al., 2013). Appropriate zinc concentrations protect against oxidative and nitrosative stress (Leonard and Maes, 2012). Preclinically, we have previously demonstrated the involvement of zinc and the GPR39 Zn\textsuperscript{2+}-sensing receptor in the pathophysiology of depressive disorders (Whittle et al., 2009; Mlyniec et al., 2013, 2014, 2015). Furthermore, suicide victims show lower expression of GPR39 in the frontal cortex and hippocampus (Mlyniec et al., 2014c), thereby highlighting the role of GPR39 in depressive behaviour and also a possible role in resistance to antidepressant treatment.

GPR39 was discovered by McKee et al., (1997) as an orphan receptor. Subsequently it was found that the GPR39 receptor belonging to the ghrelin/neurotensin subfamily is activated by zinc ions (Holst et al., 2007), which may act as a neurotransmitter in the central nervous system (Chorin et al., 2011). Activation of GPR39 triggers the G\alpha\textsubscript{q}, G\alpha\textsubscript{12/13} and G\alpha\textsubscript{s} pathways. Stimulation of the G\alpha\textsubscript{q} and G\alpha\textsubscript{12/13} pathways by zinc is enhanced by signalling, that also occurs through the G\alpha\textsubscript{s} pathway (Popovics and Stewart, 2011). Upon zinc-induced activation, an increase in CRE transcription was observed (Holst et al., 2007), which in turn led to the expression of cAMP response element-binding protein (CREB). Phosphorylation of CREB leads to transcriptional...
activation of genes, many of which are involved in depressive disorder (Marsden, 2013). We have previously shown that chronic zinc-deficient diet leads to enhanced passive-coping behaviour (Whittle et al., 2009) along with reduced BDNF, TrkB, and CREB in the hippocampus in mice (Mlyniec et al., 2014a). In a previous study, GPR39 up-regulation in the frontal cortex of mice receiving a zinc-adequate (standard) diet after chronic treatment with selective antidepressants was demonstrated (Mlyniec et al., 2013). These studies suggest that zinc via a GPR39 receptor plays a critical role in the modulation of passive-coping behaviour as well as in the antidepressant response. This is further highlighted by a previous study showing that GPR39 KO mice display enhanced passive-coping behaviour (Mlyniec et al., 2015). Furthermore, conventional antidepressants (e.g. paroxetine and imipramine) are ineffective in GPR39 KO mice, thereby indicating that GPR39 is required for the antidepressant effect of monoamine-based antidepressants (Mlyniec et al., 2015).

However, it is still unclear as to which brain regions are involved in the enhanced passive-coping phenotype in the GPR39 KO mice and its resistance to monoamine-based antidepressant treatment. Using immediate-early-gene-mapping studies, we addressed this question in the present study. Briefly, following chronic imipramine administration, we assessed the passive-coping behaviour exhibited by GPR39 KO mice. Immediate-early-gene mapping was then conducted in regions known to be involved in anxiety/passive-coping behaviour.

2. Materials and methods

2.1. Animals and treatments

GPR39 (−/−) male mice as described by (Mlyniec et al., 2015) were generated at the Faculty of Pharmacy, Jagiellonian University Medical College in Krakow, through homologous recombination by targeting the first exon of GPR39, and replacing the nucleotides from position 278–647 of the open reading frame with a neomycin-containing cassette. The chimeric males were crossed with C57BL/6 females. The mice were obtained through heterozygous breeding, resulting in wild-type, homozygous or heterozygous litters. Genotypes were verified by polymerase chain reaction methods, with the use of the following primers: forward KO: 5′ TACCAAGGTTCTCGCTCTGT, reverse WT: 5′ TACATCGATCA TACCAAGGTTCTCGCTCTGT, reverse KO: 5′ TGAATCTCGGGTTCATCTC, forward WT: 5′ TCTATCATACTCAGCCATGTT, reverse WT: 5′ ACTCGATACCCATTGCAAAG. Six-week-old GPR39 wild-type (WT; −/+) and knockout (KO; −/−) mice were housed at the Animal Facility (Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland), under standard laboratory conditions, with a natural day-night cycle, a temperature of 22 ± 2 °C and humidity at 55 ± 5%. The access to food (Altromin 1314, purchased from Animalab) as well as water was ad libitum.

10–12 week old mice were divided into four groups according to their genotype and the type of chronic drug administration (14 days) as follows: i) GPR39 WT + 0.9% NaCl; ii) GPR39 KO + 0.9% NaCl; iii) GPR39 WT + imipramine (30 mg/kg); iv) GPR39 KO + imipramine (30 mg/kg). The drugs were administered once, at 9.00 a.m., intraperitoneally (i.p.) and dissolved in 10 ml of saline (NaCl). Control mice were treated only with saline. 30 μm sections were cut using a cryostat, mounted onto gelatin-coated slides (5–6 sections/slide), and stored at −80 °C until further processing for c-Fos immunohistochemistry.

2.2. Forced swim test

The forced swim test (FST) was carried out on GPR39 KO and WT mice following chronic imipramine (30 mg/kg) or saline treatment. Mice were dropped individually into a glass cylinder containing water (Porsolt et al., 1977), which was maintained of 24 °C ± 1 °C. The temperature was measured and the water was changed before each test. The total duration of immobility after the first 2 min of adaptation time was measured during the following 4 min of the test. Prolonged immobility time in the FST suggests depressive-like behaviour, because immobility time reflects the level of despair of the mice. The camera was not switched on for 1 mouse from the GPR39 KO saline group; therefore, the immobility time could not be assessed for this particular animal. However the tissue was still further processed and analyzed as described below.

2.3. Tissue preparation

Brains were taken out 2 h after the forced swim test via cervical dislocation and rapid decapitation and shock frozen. Mice received imipramine (30 mg/kg) chronically for 14 days. The drugs were administered intraperitoneally (i.p.), dissolved in 10 ml of saline (NaCl). Control mice were treated only with saline. 30 μm sections were cut using a cryostat, mounted onto gelatin-coated slides (5–6 sections/slide), and stored at −80 °C until further processing for c-Fos immunohistochemistry.

2.4. c-Fos immunohistochemistry

The staining procedure was performed according to a previous protocol (Fitzgerald et al., 2015) with slight modifications. One level per brain region was chosen for the analysis. The whole staining procedure was conducted in boxes each containing up to 25 slides. After pre-incubation in 4% paraformaldehyde (PFA) for 40 min and a wash in phosphate buffered saline, the sections were incubated for 30 min in 1.2% H2O2. After two washes in immunobuffer (10 min/wash), the sections were incubated in 2% normal goat serum for another 30 min. Subsequently, the sections were incubated in immunobuffer containing normal goat serum (5%) and the polyclonal primary antibody (1:10,000 dilution) raised against the epitope mapping at the C-terminus of c-Fos of rabbit origin (Santa Cruz Biotechnology, Santa Cruz, CA, USA; catalogue no: sc-52) for 48 h at room temp. The primary antibody was removed and, following three rinses (10 min each), the sections were incubated in immunobuffer containing a biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, USA; 1:200) and normal goat serum (5%). The secondary antibody was removed, and the sections were washed three times with immunobuffer and incubated in avidin biotin complex (0.5%) solution for 2 h (Vectastain, ABC Kit, Vector Laboratories, Burlingame, USA). Three washes in 50 mM Tris buffer (10 min/wash) were followed by incubation of the sections in 0.05% DAB in 0.1 M phosphate buffered solution for 10 min. The chromogenic reaction (Fig. 4.1B) was started by adding H2O2 (final concentration 0.1%). Once a colour change had been observed and no further darkening of the slices was expected (after approximately 5 min), the reaction was stopped by three washes in 50 mM Tris buffer and the slides were air-dried overnight. Finally, the slides were washed in deionized water in order to remove saline residues, dehydrated in 100% ethanol for 10 min, cleared in Histoclear (Vogel-Gießener GmbH & Co.KG, Gießen, Germany) for 10 min and coverslipped in Eukitt mounting medium (Kindler, Freiburg, Germany).

2.5. Quantification of c-Fos-positive cells

The sections obtained from each brain were 3.3′-diaminobenzidine stained, and immunoreactive cells were stereologically counted using a computer-assisted image analysis system (Nikon E–800 microscope, CCD video camera, Optronics MicroFire, Goleta, CA, USA; Stereo Investigator Software, MicroBrightField Europe eK, Magdeburg, Germany) applying a 10 × magnification. A cell was considered as c-Fos-labeled (c-Fos positive) if the brown-black 3,3′-diaminobenzidine-
Neuropharmacology 198 (2021) 108752

3

stained nucleus was unambiguously darker than the background staining, and this included all cells from low to high intensities of staining. Quantification of cells was conducted in different brain structures known to be components of the fear neurocircuity, including the mPFC, the amygdala and the hippocampus, with the help of illustrations from a stereotaxic atlas (Paxinos and Franklin, 2007). The number of c-Fos-positive cells was quantified bilaterally per section of each mouse brain in a tissue area of 0.01 mm² for the medial prefrontal cortex, the nucleus accumbens, the amygdala and the CA1, CA2, CA3 while the entire area of the dentate gyrus was counted.

2.6. Data presentation and statistical analysis

Data are given as the mean number of c-Fos-positive cells per 0.01 mm² or per brain area. The data represent the mean ± the standard error of the mean (SEM). Statistical analysis was performed using STATISTICA 7.1 (Stat Soft, Inc., Tulsa, OK, USA). After all the data had been checked for outliers using Grubbs’ test, data that remained were then first tested for homogeneity using Levene’s test. A parametric distribution was revealed for the majority of the data and, thus, the number of c-Fos-positive cells was further analyzed by two-way ANOVA with genotype and treatment as the independent variables and c-Fos-positive cells as the dependent variable, followed by a post Fisher-LSD test where allowed. Statistical significance was set at \( p < 0.05 \) and a trend towards statistical significance was set at \( p < 0.09 \), as indicated in the figure and table legends in more detail.

3. Results

3.1. GPR39 KO mice displayed enhanced passive-coping behaviour

Following chronic saline/imipramine treatment the mice were subjected to the forced swim test and passive-coping behaviour was

Fig. 1. GPR39 KO mice display the pro-depressive phenotype in the forced swim test: GPR39 KO mice showed a higher immobility time (vs WT). Imipramine reduced the immobility time in both the WT and the GPR39 KO mice; however, the decrease was less pronounced in the GPR39 KO in comparison with the WT mice. ***\( p < 0.001 \), WT-sal vs WT-imi; #\( p < 0.05 \), WT-sal vs GPR39 KO-sal; $\ p < 0.05 \), GPR39 KO-sal vs GPR39 KO-imi; §§§\( p < 0.001 \), WT-imi vs GPR39 KO-imi. Sal: saline, Imi: imipramine, WT: wild type. WT sal (\( n = 8 \)), WT imi (\( n = 8 \)), KO sal (\( n = 7 \)), KO imi (\( n = 8 \)).

3.1. GPR39 KO mice displayed enhanced passive-coping behaviour

Following chronic saline/imipramine treatment the mice were subjected to the forced swim test and passive-coping behaviour was

Fig. 2. GPR39 KO mice display aberrant activity within the medial prefrontal cortex following swim-stress: GPR39 KO mice showed a higher number of c-Fos positive cells in the prelimbic (a), infralimbic (c) and cingulate cortex (b) following forced swim test. Imipramine reduced (but not significant) number of c-Fos positive cells in the prelimbic and cingulate cortex, but not the infralimbic cortex in the GPR39 KO mice. Imipramine treatment in the WT mice was associated with lower number of c-Fos positive cells in the prelimbic and cingulate cortex, but not the infralimbic cortex. d. Representative image from Paxinos and Franklin (2007) showing the regions counted. e. A schematic figure showing the regions counted. Inset shows magnified representative images from the prelimbic cortex. *\( p < 0.05 \), WT-sal vs WT-imi; #\( p < 0.05 \), WT-sal vs GPR39 KO-sal; $\ p < 0.05 \), GPR39 KO-sal vs GPR39 KO-imi. Sal: saline, Imi-imipramine, WT-wild type. Cg1: WT sal (\( n = 7 \)), WT imi (\( n = 5 \)), KO sal (\( n = 8 \)), KO imi (\( n = 8 \)); PrL: WT sal (\( n = 7 \)), WT imi (\( n = 5 \)), KO sal (\( n = 8 \)), KO imi (\( n = 8 \)); Il: WT sal (\( n = 6 \)), WT imi (\( n = 5 \)), KO sal (\( n = 8 \)), KO imi (\( n = 8 \)). Scale bar: 100 μm.
Fig. 3. GPR39 KO mice display aberrant activity within the dentate gyrus following swim-stress: GPR39 KO mice show lower number of c-Fos positive cells in anterior (Fig a), but not posterior (Fig b), dentate gyrus following forced swim test. Imipramine induced reduction in immobility time in both the genotypes was associated with reduction in the c-Fos positive cells. Representative picture from each group (c and d). ***p < 0.05, WT-sal vs WT-imi; ##p < 0.01, WT-sal vs GPR39 KO-sal; $ p < 0.05, GPR39 KO-sal vs GPR39 KO-imi. Sal: saline, Imi-imipramine, WT-wild type. Ant. DG: WT sal (n = 8), WT imi (n = 7), KO sal (n = 8), KO imi (n = 8); Post DG: WT sal (n = 8), WT imi (n = 6), KO sal (n = 8), KO imi (n = 8). Scale bar: 100 μm.

Fig. 4. GPR39 KO mice display aberrant activity within the hippocampal subregions following swim-stress: GPR39 KO mice showed a higher number of c-Fos positive cells in the dorsal CA1, CA2 and CA3 region following forced swim test (Fig a, b, c). Imipramine reduced (but not always significant) number of c-Fos positive cells in these regions in the GPR39 KO mice. Imipramine in the WT mice increased the number of c-Fos positive cells in the anterior CA2 and CA3, but not CA1 region. c-Fos positive cells in posterior CA1 and CA3, but not CA2, region remain unchanged (Fig d, e, f). Representative pictures from Paxinos and Franklin (2007) showing anterior and posterior regions of the hippocampus and the areas counted (marked in red rectangle) (Fig g and h) ***p < 0.05, WT-sal vs WT-imi; #p < 0.05, ##p < 0.01, ###p < 0.001, WT-sal vs GPR39 KO-sal; $ p < 0.05, GPR39 KO-sal vs GPR39 KO-imi. Sal: saline, Imi-imipramine, WT-wild type. Ant. CA1: WT sal (n = 8), WT imi (n = 7), KO sal (n = 8), KO imi (n = 8); Ant. CA2: WT sal (n = 8), WT imi (n = 7), KO sal (n = 8), KO imi (n = 8); Ant. CA3: WT sal (n = 8), WT imi (n = 7), KO sal (n = 8), KO imi (n = 8); Post. CA1: WT sal (n = 8), WT imi (n = 6), KO sal (n = 7), KO imi (n = 8); Post. CA2: WT sal (n = 8), WT imi (n = 6), KO sal (n = 7), KO imi (n = 8); Post. CA3: WT sal (n = 6), WT imi (n = 6), KO sal (n = 7), KO imi (n = 8). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
evaluated using immobility time as the index. There was a significant genotype × drug effect \((F_{(1,27)} = 6.5, p < 0.05)\). Post hoc analysis revealed that GPR39 KO mice displayed a higher immobility time in comparison with WT mice \((p < 0.05)\), reflecting an enhanced passive-coping behaviour as also shown in our previous experiments. Also as shown before, chronic imipramine reduced the immobility time in the WT mice \((p < 0.001)\). Imipramine also reduced the immobility time in the GPR39 KO mice \((p < 0.05)\). However, imipramine-induced reduction in immobility time in the GPR39 KO mice was significantly less \((around 10\%)\) than it was for WT mice \((around 30\%)\) (Fig. 1).

3.2. **GPR39 KO mice displayed a hyperactive medial prefrontal cortex following swim stress**

-c-Fos was used as a surrogate marker for neuronal activity following the forced swim test. There was a significant genotype \((F_{(1,24)} = 10.72, p < 0.01)\) and treatment \((F_{(1,24)} = 8.63, p < 0.01)\) effect but no genotype × drug effect within the prelimbic cortex (PrL). Post hoc test further revealed that saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.05)\). Imipramine treatment reduced c-Fos expression both in the WT \((p < 0.05, vs WT\) saline) as well as GPR39 KO mice \((p < 0.05, vs KO\) saline) (Fig. 2a and e). These findings parallel the behavioural effect wherein a partial reduction in imipramine induced immobility time is associated with a partial reduction in c-Fos expression within the PrL in the GPR39KO mice as compared to the WT mice.

A similar picture emerged in the Cg1. There was a significant genotype \((F_{(1,24)} = 11.04, p < 0.01)\) and treatment \((F_{(1,24)} = 7.72, p = 0.01)\) effect but no genotype × drug effect within the Cg1. Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.05)\). Imipramine treatment reduced c-Fos expression both in the WT \((p < 0.05, vs WT\) saline) as well as GPR39 KO mice \((p < 0.05, vs KO\) saline) (Fig. 2b). There was a significant genotype \((F_{(1,23)} = 12.75, p < 0.01)\) effect within the infralimbic cortex (IL). Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.01)\). Unlike the Cg1 or PrL, imipramine did not normalize the enhanced c-Fos expression within the GPR39 KO mice (Fig. 2c).

3.3. **GPR39 KO mice displayed a hypooactive anterior dentate gyrus following swim stress**

There was a significant genotype \((F_{(1,27)} = 15.16, p < 0.001)\) and treatment \((F_{(1,27)} = 25.53, p < 0.001)\) effect but no genotype × drug effect within the anterior dentate gyrus (DG). Post hoc test further revealed that saline treated GPR39 KO mice displayed lower c-Fos expression in comparison to the WT mice \((p < 0.01)\). Imipramine treatment reduced c-Fos expression both in the WT \((p < 0.01, vs WT\) saline) as well as GPR39 KO mice \((p < 0.05, vs KO\) saline) (Fig. 3a and c). Unlike the anterior DG, no differences were observed in c-Fos expression in the posterior DG (Fig. 3b and d).

3.4. **GPR39 KO mice displayed a hyperactive hippocampus following swim stress**

There was nearly a significant genotype × drug effect \((F_{(1,27)} = 3.68, p = 0.06)\) within the anterior CA1. Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.05)\). Imipramine treatment reduced c-Fos expression both in GPR39 KO mice \((p < 0.05, vs KO\) saline) but not in the WT mice (Fig. 4a).

Within the anterior CA2, again a significant genotype × drug effect \((F_{(1,27)} = 7.96, p < 0.01)\) was observed. Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.001)\). Imipramine treatment increased c-Fos expression within the WT mice \((p < 0.05, vs WT\) saline), while it reduced the c-Fos expression in the GPR39 KO mice \((p = 0.08, vs KO\) saline) (Fig. 4b).

Within the anterior CA3, again a significant genotype × drug effect \((F_{(1,27)} = 7.62, p < 0.05)\) was observed. Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.01)\). Imipramine treatment increased c-Fos expression within the WT mice \((p < 0.05, vs WT\) saline), while it did not affect the c-Fos expression in the GPR39 KO mice \((p > 0.05, vs KO\) saline) (Fig. 4c).

Unlike the anterior hippocampus wherein c-Fos expression changes were observed in all 3 regions, within the posterior hippocampus CA2 emerged as the most interesting region. A significant genotype × drug effect \((F_{(1,23)} = 7.78, p < 0.05)\) was observed within the posterior CA2. Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.01)\). Imipramine treatment reduced c-Fos expression within the GPR39 KO mice \((p < 0.05, vs KO\) saline) without affecting the c-Fos expression in the WT mice (Fig. 4e).

3.5. **GPR39 KO mice displayed a hyperactive posterodorsal division of the medial amygdala following swim stress**

There was a significant genotype × drug effect \((F_{(1,26)} = 4.36, p = 0.05)\) posterodorsal division of the medial amygdala (MePD). Saline treated GPR39 KO mice displayed higher c-Fos expression in comparison to the WT mice \((p < 0.05)\). Imipramine treatment reduced c-Fos expression in GPR39 KO mice \((p < 0.01, vs KO\) saline) but not in the WT mice \((p > 0.05, vs WT\) saline) (Fig. 5a and c).

4. Discussion

In this study, first we confirmed previous results showing that mice lacking the Zn2+ sensing receptor GPR39 display enhanced passive-coping behaviour compared with their WT controls. In addition, the present study shows for the first time that GPR39 is associated with modulation of neuronal activity in key brain regions (such as the mPFC, the hippocampus and the amygdalar subregion) associated with passive-coping behavior (Table 1). Furthermore, our data suggest that monoamine-based antidepressants such as imipramine exert their action via GPR39-dependent and -independent pathways. Failure to modulate depression-related aberrant activity in important brain areas of the depression circuitry is proposed to mediate/contribute to the greatly reduced antidepressant action of monoamine-based antidepressants in GPR39 KO mice. The failure to normalize enhanced passive-coping related aberrant activity in the above-mentioned candidate brain regions (which are important in the depression circuit) could play an important role in mediating/contributing to the greatly reduced anti-depressant action of monoamine-based antidepressants in GPR39 KO mice.

4.1. **GPR39 KO mice display enhanced passive-coping behaviour, which is marginally normalised by imipramine**

The behavioural results in this study confirm previous observations demonstrating that GPR39 KO mice elicit a pro-depressive phenotype in comparison with their WT counterparts (Mlyniec et al., 2014a, 2015). Furthermore, GPR39 KO mice also display enhanced anxiety-like behaviour (Mlyniec et al., 2015). Together, these studies suggest GPR39 KO mice as a putative animal model of enhanced anxiety and passive-coping behaviour. In contrast to the effect of imipramine in WT mice, wherein a significant reduction in immobility time was observed (around 30%), imipramine in GPR39 KO could only partially rescue the pro-depressive phenotype (by around 10%). These observations indicate that the absence of the GPR39 receptor might account for the greatly reduced antidepressant action of imipramine in the KO mice. In addition, it is possible too that GPR39-independent mechanisms might also play an important role in the action of these antidepressants. It is noteworthy here that NMDA receptor antagonists can still exert their efficacy in the GPR39 KO mice (Mlyniec et al., 2015), thereby suggesting that GPR39 expression is not critical for all classes of antidepressants.
The enhanced passive-coping behaviour demonstrated in GPR39 KO mice following swim stress was associated with differential neuronal activation in 10 out of 22 investigated areas known to be involved in stress regulation and the modulation of mood.

4.2. GPR39 KO mice display aberrant activity in key brain regions associated with anxiety/depression

**Ventromedial prefrontal cortex (vmPFC):** compared with WT controls, GPR39 KO mice showed enhanced neuronal activity in the medial prefrontal cortex, including the prelimbic (PL) and the cingulate (Cg) cortices. These observations are in line with clinical findings as enhanced mPFC activity is found in human PET studies that, likewise, demonstrate higher metabolic activity in response to negative emotional stimuli (e.g. sad faces) in the Brodman area 25 (Mayberg et al., 1999; Seminowicz et al., 2004), the most commonly suggested anatomic correlate of the mPFC in humans (Takagishi and Chiba, 1991; Gabbott et al., 2003).

Chronic imipramine reduced both the immobility time in the WT mice and the activity within the mPFC. On the other hand, imipramine induced a marginal reduction of enhanced passive-coping behaviour in the GPR39 KO mice. Furthermore, human patients, decreased BA25 activity has been associated with successful treatment using antidepressant drugs (Mayberg et al., 2000; Keedwell et al., 2009); also see Delaveau et al., 2011), electroconvulsive therapy (Nobler et al., 2001; Suwa et al., 2012), repetitive transcranial stimulation (Mottaghy et al., 2002; Kito et al., 2012), ablative surgery (Dougherty et al., 2003) and deep brain stimulation in humans (Mayberg et al., 2005).

**Hippocampus:** GPR39 KO mice showed hypoactivation in the anterior, but not the posterior, dentate gyrus (DG). This finding is in line with previous observations in the HAB/NAB model in response to swim stress (Sah et al., 2012) and open arm exposure (Muigg et al., 2009). Chronic imipramine reduced both the immobility time in the WT mice and the activity within the mPFC. On the other hand, imipramine induced a marginal reduction of enhanced passive-coping behaviour in the GPR39 KO mice. Furthermore, imipramine failed to normalize the hyperactivity in the infralimbic cortex and elicited only partial normalization of the hyperactive cingulate and prelimbic cortices. Failure to elicit normalization of aberrant activity within these subregions of the medial prefrontal cortex might account for the marginal/reduced antidepressant effect elicited by imipramine. These data further show that GPR39 within the mPFC is important both for passive-coping behaviour and (marginally) for the antidepressant response. Normalization of the hyperactive mPFC activity following a successful antidepressant response has previously been observed in preclinical studies (Muigg et al., 2007). Furthermore, in human patients, decreased BA25 activity has been associated with successful treatment using antidepressant drugs (Mayberg et al., 2000; Keedwell et al., 2009); also see Delaveau et al., 2011), electroconvulsive therapy (Nobler et al., 2001; Suwa et al., 2012), repetitive transcranial stimulation (Mottaghy et al., 2002; Kito et al., 2012), ablative surgery (Dougherty et al., 2003) and deep brain stimulation in humans (Mayberg et al., 2005).

Chronic imipramine reduced both the immobility time in the WT mice and the activity within the mPFC. On the other hand, imipramine induced a marginal reduction of enhanced passive-coping behaviour in the GPR39 KO mice. Furthermore, imipramine failed to normalize the hyperactivity in the infralimbic cortex and elicited only partial normalization of the hyperactive cingulate and prelimbic cortices. Failure to elicit normalization of aberrant activity within these subregions of the medial prefrontal cortex might account for the marginal/reduced antidepressant effect elicited by imipramine. These data further show that GPR39 within the mPFC is important both for passive-coping behaviour and (marginally) for the antidepressant response. Normalization of the hyperactive mPFC activity following a successful antidepressant response has previously been observed in preclinical studies (Muigg et al., 2007). Furthermore, in human patients, decreased BA25 activity has been associated with successful treatment using antidepressant drugs (Mayberg et al., 2000; Keedwell et al., 2009); also see Delaveau et al., 2011), electroconvulsive therapy (Nobler et al., 2001; Suwa et al., 2012), repetitive transcranial stimulation (Mottaghy et al., 2002; Kito et al., 2012), ablative surgery (Dougherty et al., 2003) and deep brain stimulation in humans (Mayberg et al., 2005).

The enhanced passive-coping behaviour demonstrated in GPR39 KO mice following swim stress was associated with differential neuronal activation in 10 out of 22 investigated areas known to be involved in stress regulation and the modulation of mood.

4.2. GPR39 KO mice display aberrant activity in key brain regions associated with anxiety/depression

**Ventromedial prefrontal cortex (vmPFC):** compared with WT controls, GPR39 KO mice showed enhanced neuronal activity in the medial prefrontal cortex, including the prelimbic (PL) and the cingulate (Cg) cortices. These observations are in line with clinical findings as enhanced mPFC activity is found in human PET studies that, likewise, demonstrate higher metabolic activity in response to negative emotional stimuli (e.g. sad faces) in the Brodman area 25 (Mayberg et al., 1999; Seminowicz et al., 2004), the most commonly suggested anatomic correlate of the mPFC in humans (Takagishi and Chiba, 1991; Gabbott et al., 2003).

Chronic imipramine reduced both the immobility time in the WT mice and the activity within the mPFC. On the other hand, imipramine induced a marginal reduction of enhanced passive-coping behaviour in the GPR39 KO mice. Furthermore, human patients, decreased BA25 activity has been associated with successful treatment using antidepressant drugs (Mayberg et al., 2000; Keedwell et al., 2009); also see Delaveau et al., 2011), electroconvulsive therapy (Nobler et al., 2001; Suwa et al., 2012), repetitive transcranial stimulation (Mottaghy et al., 2002; Kito et al., 2012), ablative surgery (Dougherty et al., 2003) and deep brain stimulation in humans (Mayberg et al., 2005).

Chronic imipramine reduced both the immobility time in the WT mice and the activity within the mPFC. On the other hand, imipramine induced a marginal reduction of enhanced passive-coping behaviour in the GPR39 KO mice. Furthermore, imipramine failed to normalize the hyperactivity in the infralimbic cortex and elicited only partial normalization of the hyperactive cingulate and prelimbic cortices. Failure to elicit normalization of aberrant activity within these subregions of the medial prefrontal cortex might account for the marginal/reduced antidepressant effect elicited by imipramine. These data further show that GPR39 within the mPFC is important both for passive-coping behaviour and (marginally) for the antidepressant response. Normalization of the hyperactive mPFC activity following a successful antidepressant response has previously been observed in preclinical studies (Muigg et al., 2007). Furthermore, in human patients, decreased BA25 activity has been associated with successful treatment using antidepressant drugs (Mayberg et al., 2000; Keedwell et al., 2009); also see Delaveau et al., 2011), electroconvulsive therapy (Nobler et al., 2001; Suwa et al., 2012), repetitive transcranial stimulation (Mottaghy et al., 2002; Kito et al., 2012), ablative surgery (Dougherty et al., 2003) and deep brain stimulation in humans (Mayberg et al., 2005).
indicates p < 0.05 but >0.01. Statistical significance was set at p < 0.05; IL = infralimbic cortex, PL = prelimbic cortex, Cg = cingulate cortex, NAcC = nucleus accumbens CeM = centromedial amygdaloid nucleus, CeC = centrocapsular amygdaloid nucleus, CeL = central lateral amygdaloid nucleus, BA = basolateral amygdaloid nucleus, LaDL = dorsolateral lateral amygdaloid nucleus, MePV = posteroverentral medial amygdaloid nucleus, MePD = posterodorsal medial amygdaloid nucleus, Aco = anterior cortical amygdaloid area, DG = dentate gyrus, CA = cornu ammonis.

Brain region WT-sal WT-imi KO-sal KO-imi Effect

Cingulate cortex 91.4 ± 1.4 66.3 ± 2.9 117.1 ± 2.4 96.3 ± 7.9 abc

IL 82.3 ± 2.2 71.2 ± 2.1 139.1 ± 1.3 115.3 ± b

PL 106.4 ± 3.2 79.6 ± 2.1 137.4 ± 1.1 100.0 ± abc

Motor cortex 75.4 ± 2.2 84.3 ± 2.1 108.7 ± 1.2 108.8 ± b

Sstriatum (± 1.70 mm) NAc core 18.2 ± 2.8 15.8 ± 2.2 19.7 ± 2.0 17.5 ± 1.8

NAcc shell 36.3 ± 5.2 36.9 ± 3.1 34.9 ± 3.4 33.1 ± 4.7

Amygdala (±1.58 mm) La DL 37.9 ± 3.7 27.7 ± 2.7 41.1 ± 4.6 36.8 ± 2.9

BA 117.0 ± 5.0 86.9 ± 4.9 122.2 ± 10.5 ±

CeM 3.7 ± 0.8 4.3 ± 0.7 4.1 ± 0.6 4.7 ± 0.6

Cel. 4.3 ± 1.1 3.3 ± 0.5 4.6 ± 1.4 4.4 ± 0.6

BMA 170.9 ± 162.0 200.3 ± 166.3 ±

26.0 20.2 16.3 18.4

MePD 52.4 ± 5.5 50.1 ± 4.0 69.8 ± 7.7 47.3 ± 6.7 bc

MePV 37.9 ± 3.7 27.7 ± 2.7 41.1 ± 4.6 36.8 ± 3.9

Aco 49.3 ± 7.3 45.9 ± 6.0 59.4 ± 6.0 44.8 ± 4.9

Hippocampus anterior (±4.58 mm) CA1 anterior 18.4 ± 1.6 18.9 ± 4.1 25.3 ± 2.1 16.6 ± 1.3 bc

CA2 anterior 8.1 ± 1.9 13.2 ± 1.5 16.8 ± 1.5 12.9 ± 1.5 abc

CA3 anterior 13.6 ± 1.2 19.4 ± 2.9 22.6 ± 1.4 18.4 ± 1.7 ab

DG anterior 23.5 ± 1.8 22.9 ± 1.1 14.9 ± 2.4 8.3 ± 1.0 abc

Hippocampus posterior (±2.70 mm) CA1 Posterior 18.8 ± 3.7 26.1 ± 2.7 26.3 ± 2.1 19.6 ± 2.9

CA2 posterior 12.6 ± 1.4 16.8 ± 2.7 21.4 ± 3.0 13.8 ± 1.8 bc

CA3 posterior 14.7 ± 2.7 21.4 ± 2.9 26.7 ± 3.0 16.5 ± 2.3

DG posterior 16.4 ± 2.7 14.3 ± 1.3 13.3 ± 1.8 9.6 ± 1.1

Moreover, GPR39 KO mice show reduced CREB/BDNF in the hippocampus (Mlyniec et al., 2014a) and suicide victims show reduced GPR39 expression in the hippocampus (Mlyniec et al., 2014c). Therefore, the aberrant network activity within the hippocampus of the GPR39 KO mice might be associated with the reduced release of neurotrophic factor activity, leading to a pro-depressive phenotype.

Chronic imipramine failed to normalize the dysregulated activity in the GPR39 KO mice within the DG and CA3 regions. Along with others using different animal models and interventions, we have consistently shown in previous studies that normalization of the blunted activity within the hippocampus, especially within the dentate gyrus, is accompanied by an antidepressant-like effect (Dagyte et al., 2010; Sah et al., 2012; Schumckermair et al., 2013; Stanisavljevic et al. 2019, 2020). Therefore, the present finding is in accordance with previous studies suggesting that a successful antidepressant-like effect involves normalization of dysregulated hippocampal activity. Furthermore, it also shows that imipramine requires GPR39 within the hippocampus to mediate its antidepressant efficacy.

Amygda: The amygdala shows one of the highest expressions of GPR39 mRNA. The amygdalar subnuclei with pronounced GPR39 expression include the lateral, basolateral, anterior, cortical and post-ero-medial cortical nuclei (Jackson et al., 2006). Since the amygdala plays an important role in anxiety/pasive-coping behaviour, we expected dysregulated activity within several nuclei following GPR39 ablation. Contrary to our expectation, we found dysregulated activity only within the posterodorsal division of the medial amygdala. In line with the present study, c-Fos expression was increased in the medial amygdala in response to acute stressors (Chen and Herbert, 1995; Dayas et al., 1999; Salchner and Singewald, 2002; Figueiredo et al., 2003) or the medial amygdala showed enhanced c-Fos responsiveness to an acute stressor (Ebner et al., 2004).

Anterograd tracing experiments have shown that axons from MeA neurons stream path and partially through the medioal aspect of the CeA (Gray et al., 1989) and project to the PVN, thereby regulating the HPA axis. As a result, GPR39 KO-induced hyperactivity of MeA might therefore also alter the PVN activity leading to the pro-depressive phenotype. This needs to be further assessed.

The hyperactivity within the posterodorsal division of the medial amygdala was normalized despite only a marginal antidepressant response, indicating that imipramine mediates its effect independently of GPR39 within the amygdala. Since various subregions of the limbic system (including the mPFC, the amygdala and the hippocampus) have been shown to be involved in anxiety/pasive-coping behaviour as well as with antidepressant responses (for a review, see (Fava and Kendler, 2000)), our data indicate that normalization of the aberrant activity in just one subregion (the amygdala in the present study) is not enough to elicit a monoamine-based successful antidepressant response. One caveat of the present study is that c-Fos could also be expressed in some glial cells besides neurons. Therefore, future studies should take this parameter into account.

In conclusion, the present data show for the first time that GPR39 is associated with modulation of neuronal activity in key brain regions (such as the mPFC, the hippocampus and the amygdalar subregion) associated with anxiety/depressive disorder. Our data also suggest that monoamine-based antidepressants such as imipramine exert their action via GPR39-dependent and -independent pathways. Failure to modulate depression-related aberrant activity in important brain areas of the depression circuitry is proposed to mediate/contribute to the greatly reduced antidepressant action of monoamine-based antidepressants in GPR39 KO mice.

CRediT authorship contribution statement

Anupam Sah: Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing. Maria Kharitonova: Investigation. Katarzyna Mlyniec: Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgements

This study was partially supported by a grant from the National Science Centre (contract DEC-2011/03/B/NZ7/01999) and by the Funds for Statutory Activity of the Jagiellonian University Medical College in Krakow, Poland.

References

Chen, X., Herbert, J., 1995. Regional changes in c-fos expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothymic and endocrine responses. Neuroscience 64 (3), 675–685.

Chorin, E., Vinograd, O., Fleidervish, I., Gilad, D., Herrmann, S., Sekler, I., Aizenman, E., Hershfinkel, M., 2011. Upregulation of KCC2 activity by zinc-mediated neurotransmission via the mNMDA/GPR39 receptor. J. Neurosci. 31 (36), 12916–12926.

Dagyte, G., Trentani, A., Postema, F., Luiten, P.G., Den Boer, J.A., Gabriel, C., Mocaer, E., Meerlo, P., Van der Zee, E.A., 2010. The novel antidepressant agonist methamphetamine normalizes hippocampal neuronal activity and promotes neurogenesis in chronically stressed rats. CNS Neurosci. Ther. 16 (4), 195–207.

Dayas, C.V., Buller, K.M., Day, T.A., 1999. Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. Eur. J. Neurosci. 11 (7), 2312–2322.
Dougherty, D.D., Weiss, A.P., Cosgrove, G.R., Alpert, N.M., Cassem, E.H., Nierenberg, A.A., Price, B.H., Mayberg, H.S., Fischman, A.J., Rauch, S.L., 2003. Cerebral metabolic correlates as potential predictors of response to anterior cingulotomy for treatment of major depression. J. Neurosurg. 96 (6), 1010–1017.

Ehlers, K., Rupniak, N.M., Sarria, A., Singewald, N., 2004. Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. Proc. Natl. Acad. Sci. U. S. A. 101 (12), 4280–4285.

Faselov, M.S., Dong, H.W., 2010. Are the dorsal and ventral hippocampal functions differentially structured? Neurosci. Lett. 53 (1–2), 59–71.

Gray, T.S., Carney, M.E., Magnussen, D.J., 1989. Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: possible role in stress-induced adrenocorticotropic release. Neuroendocrinology 50 (4), 433–446.

Holst, B., Egerod, K.L., Schild, E., Vickers, S.P., Ocheraen, S., Gerlach, L.O., Storjohann, L., Stueden, C.E., Jones, R., Beck-Sickinger, A.G., Schwartz, T.W., 2007. GPCR signaling is stimulated by zinc ions but not by estriol. Endocrinology 148 (1), 13–20.

Jackson, V.B., Notcharber, H.P., Civelli, O., 2006. GPR39 receptor expression in the mouse brain. Neuroreport 17 (8), 813–816.

Keedwell, P., Drapej, D., Surguladze, S., Giampietra, V., Bremmer, M., Phillips, M., 2009. Neural markers of symptomatic improvement during antidepressant therapy in severe depression: subgenual cingulate and visual cortical responses to sad, but not happy, facial stimuli are correlated with changes in symptom score. J. Psychopharmacol. 23 (7), 775–788.

Kingwell, K., 2010. Depression: in pursuit of happiness. Nat. Rev. Neurosci. 11 (12), 786. Kito, S., Hasegawa, T., Koga, Y., 2012. Cerebral blood flow in the ventromedial prefrontal cortex correlates with treatment response to low-frequency repetitive transcranial magnetic stimulation in the treatment of depression. Psychiatr. Clin. Neurosci. 66 (2), 138–145.

Lav, J., Barsova, V., Belszun, C., Surget, A., 2016. Decline of hippocampal stress reactivity and neuronal ensemble coherence in a mouse model of depression. Psychoneuroendocrinology 67, 113–123.

Leonard, B., 2012. Mechanistic explanations how cell-mediated immune regulation in the hippocampus. Int. J. Neuropsychopharmacol. 18 (3).

Mayberg, H.S., Brannan, S.K., Tekell, J.L., Silva, J.A., Mahurin, R.K., McGinnis, S., Law, J., Ibarguen-Vargas, Y., Belzung, C., Surget, A., 2016. Decline of hippocampal stress activation, inflammation and oxidative and nitrosative stress pathways and their functional correlates. Prog. Neuro-psychopharmacol. Biol. Psychiatry 43, 168–184.

Mlyniec, K., Davies, C.L., de Aguero Sanchez, I.G., Pytka, K., Budziszewska, B., Singewald, N., 2014b. Essential elements in depression and anxiety. Part I. PharmacoL Rep. 66 (4), 534–544.

Mlyniec, K., Budziszewska, B., Szewczyk, B., Sowa-Kucma, M., Misztak, P., Piekoszowski, W., Trela, F., Ostachowicz, B., Nowak, G., 2014c. The involvement of the GPR39-Zn(2+)–sensing receptor in the pathophysiology of depression. Studies in rodent models and suicide victims. Neuropharmacology 79, 290–297.

Mlyniec, K., Singewald, N., Holst, B., Nowak, G., 2015. GPR39 Zn(2+)–sensing receptor: a new target in antidepressant development? J. Affect. Disord. 174, 89–100.

Mottaghy, F.M., Keller, C.E., Gangitano, M., Ly, J., Thill, M., Parker, J.A., Pascual-Leone, A., 2002. Correlation of cerebral blood flow and treatment effects of repetitive transcranial magnetic stimulation in depressed patients. Psychiatr. Res. 115 (1–2), 1–14.

Muigg, P., Hoeld, U., Pfaffrader, K., Neumann, L., Landgraf, R., Singewald, N., 2007. Altered brain activation pattern associated with drug-induced attenuation of enhanced depression-like behavior in rats bred for high anxiety. Biol. Psychiatr. 61 (6), 782–796.

Muigg, P., Scheiber, S., Salchner, P., Bunck, M., Landgraf, R., Singewald, N., 2009. Differential stress-induced neuronal activation patterns in mouse lines selectively bred for high or low anxiety. PloS One 4 (4), e538.

Nobler, M.S., Oquendo, M.A., Kegeles, L.S., Malone, K.M., Campbell, C.C., Sackeim, H.A., Mann, J.J., 2001. Decreased regional brain metabolism after electroconvulsive therapy. Am. J. Psychiatr. 158 (2), 305–308.

Paxinos, G., Franklin, K., 2007. The Mouse Brain in Stereotaxic Coordinates, Third Edition. Elsevier.

Popovic, P., Stewart, A.J., 2011. GPR39: a Zn(2+)-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. Cell. Mol. Life Sci. 68 (1), 85–95.

Porsolt, R.D., Bertin, A., Jalife, M., 1979. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229 (2), 327–336.

Sah, A., Schemmernair, C., Sartori, S.H., Gaburro, S., Kasandasy, M., Irshick, R., Klimaschewski, L., Landgraf, R., Aigner, L., Singewald, N., 2012. Anxiety- rather than depression-like behavior is associated with adult neurogenesis in a female mouse model of higher trait anxiety- and comorbid depression-like behavior. Transl. Psychiatry 2, e171.

Salchner, P., Singewald, N., 2002. Neuroanatomical substrates involved in the anxious-like effect of acute fluoxetine treatment. Neuropharmacology 43 (8), 1238–1248.

Schmuckermair, C., Gaburro, S., Sah, A., Landgraf, R., Sartori, S.B., Singewald, N., 2013. Behavioral and neurobiological effects of deep brain stimulation in a mouse model of high anxiety- and depression-like behavior. Neuropsychopharmacology 38 (7), 1324–1334.

Seminowicz, D.A., Mayberg, H.S., McIntosh, A.R., Goldapple, K., Kennedy, S., Segal, Z., Raffi-Tari, S., 2004. Limbic-frontal circuitry in major depression: a path modeling metaanalysis. Neuroimage 22 (1), 409–418.

Sievek, M., Szewczyk, B., Dudek, D., Styzenek, K., Sowa-Kucma, M., Mlyniec, K., Sievek, A., Witkowski, L., Pochwist, B., Nowak, G., 2013. Zinc as a marker of affective disorders. PharmacoL Rep. 65 (6), 1512–1518.

Stanisavljevic, A., Peric, I., Gass, P., Inta, D., Lang, U.E., Borgwardt, S., Filipovic, D., 2019. Brain sub-region-specific effects of clonazepam on c-fos expression of chronically socially isolated rats. Neuroscience 428, 1046–1058.

Sowa, T., Namiki, C., Takaya, S., Oshita, A., Ishina, K., Fukuyama, H., Suga, H., Murai, T., 2012. Corticobasal balance shift of regional glucose metabolism in depressed patients treated with ECT. J. Affect. Disord. 136 (3), 1039–1046.

Szwajdzik, W., Herrmann, N., Mazereeuw, G., Goldberg, K., Harimoto, T., Lancot, K.L., 2013. Zinc in depression: a meta-analysis. Biol. Psychiatr. 74 (12), 872–878.

Takagi, M., Chiba, T., 1991. Differential effects of the intrahippocampal (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. Brain Res. 566 (1–2), 26–34.

Tomar, A., Politygow, D., Chattarjee, S., McIgh, T.J., 2015. The dynamic impact of repeated stress on the hippocampal spatial map. Hippocampus 25 (1), 38–50.

Whittle, N., Lubec, G., Singewald, N., 2009. Zinc deficiency induces enhanced depression-like behaviour and altered limbic activation reversed by antidepressant treatment in mice. Amino Acids 36 (1), 147–158.