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Chronic venlafaxine treatment fails to alter the levels of galanin system transcripts in normal rats

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ABSTRACT

It is widely accepted that efficacy and speed of current antidepressants' therapeutic effect are far from optimal. Thus, there is a need for the development of antidepressants with new mechanisms of action. The neuropeptide galanin and its receptors (GalR1, GalR2 and GalR3) are among the promising targets. However, it is not clear whether or not the galanin system is involved in the antidepressant effect exerted by the currently much used inhibitors of the reuptake of serotonin and/or noradrenaline. To answer this question we administered the selective serotonin and noradrenaline reuptake inhibitor (SNRI) venlafaxine (40 mg/kg/day via osmotic minipumps) to normal rats and examined the levels of the transcripts for galanin and GalR1–3 after a 3-week venlafaxine treatment in the dorsal raphe, hippocampus and frontal cortex. These areas are known to be involved in the effects of antidepressants and in depression itself. Venlafaxine failed to alter the expression of any of the galanin system genes in these areas. Our results show that one of the most efficient, currently used SNRIs does not alter transcript levels of galanin or its three receptors in normal rats. These findings suggest that the pro- and antidepressive-like effects of galanin reported in animal experiments may employ a novel mechanism(s).

1. Introduction

Major depressive disorder (MDD) is amongst the most debilitating diseases of modern societies (Han and Wang, 2005; Kessler et al., 2006). While pharmacological treatments do exist, they lack effectiveness in many individuals (O'Leary et al., 2015; Pigott et al., 2010; Thase et al., 2005; Trivedi et al., 2006), they cannot always prevent further depressive episodes (Pigott et al., 2010; Trivedi et al., 2006) and they have a delay in the onset of their therapeutic effect (Gex-Fabry et al., 2004; Machado-Vieira et al., 2008; O'Leary et al., 2015; Smith et al., 2002). Thus, research is ongoing for discovering better antidepressant treatments and new possible targets (O'Leary et al., 2015). In these efforts the galanin system has emerged as a candidate for further exploration (Counts et al., 2008; Elvander et al., 2004; Holets et al., 1988; Lang et al., 2015; Pieribone et al., 1998; Senut et al., 1989; Skofitsch et al., 1986; Weiss et al., 1998; Xu and Hokfelt, 1997).

Galanin is a 29 amino acid long neuropeptide (Tatemoto et al., 1983) acting via three G-protein coupled receptors, Gal1, Gal2 and Gal3 (Barreda-Gomez et al., 2014; Branchek et al., 2000; Lang et al., 2015). Galanin and the Gal1–3 receptors are widely distributed in the rat (Melander et al., 1986; O'Donnell et al., 2002; Skofitsch and Jacobowitz, 1985; Skofitsch et al., 1986) and human (Kohler et al., 1989; Kordower et al., 1992) brain, and among their many functions is the modulation of the brain's serotonin (5-HT) and noradrenaline (NA) neurons (Fuxe et al., 1998; Pieribone et al., 1998; Weiss et al., 1998). This was further supported by the fact that all three receptors are found in the dorsal raphe (DR) and locus coeruleus (LC) of rats (Burazin et al., 2000; Mennicken et al., 2002; O'Donnell et al., 1999, 2002) and that galanin is co-expressed in almost 40% of the serotonergic neurons in the DR (Xu and Hokfelt, 1997) and in around 80% of the noradrenergic neurons in the LC (Holets et al., 1988). However, reports indicate that galanin also modulates GABAergic (Sharkey et al., 2008) and cholinergic neurotransmission (Counts et al., 2008; Elvander et al., 2004; Senut et al., 1989) and may thus directly and indirectly influence functions in distinct regions, such as the hippocampus (HC) and frontal cortex (FC). Functionally, galanin has been suggested to be implicated in numerous pathological states and physiological processes in the central

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nervous system, like learning and memory (Brewer et al., 2005), epilepsy (Kovac and Walker, 2013; Lerner et al., 2008), addiction (Picciotto, 2010) or pain perception (Xu et al., 2008), but particular focus has been on depressive states and anxiety-related disorders/behaviors (Fuxe et al., 1998; Kuteeva et al., 2008a, 2008b; Lu et al., 2007; Murck et al., 2004). Indeed, studies showed that a Gal3 antagonist (Swanson et al., 2005) and a Gal2 agonist (Kuteeva et al., 2008b; Lu et al., 2005; Saar et al., 2013a, 2013b) attenuated depressive symptoms in rodent models of depression, while recent association studies by Wray et al. (2012) and Juhasz et al. (2014) have underlined the importance of polymorphisms in the galanin genes in humans. However, increase in galanin release may exert opposite effects on depression related phenotypes. In general terms, reports cited above indicate that activation of Gal1 and Gal3 causes depression-like effects, while blockade of these receptors and activation of Gal2 were associated with antidepressant-like effects in the behavior of rats.

The galanin-like peptide precursor (Galp) encodes two transcripts related to the galanin system which are formed through splicing. One, Galp, was isolated from porcine hypothalamus and considered originally as a possible Gal2 agonist (Ohtaki et al., 1999). Later its agonistic properties have also been demonstrated for Gal1 and Gal3 in transfected cells, and results from knock-out mice raised the possibility of a so far undiscovered native receptor for the peptide (Krasnow et al., 2004; Lang et al., 2005, 2015). The expression of Galp is restricted to specific cells in the hypothalamus, its release is regulated by e.g. insulin and leptin (Jureus et al., 2000, 2001) and once released, it influences feeding behavior and thermogenesis (Hansen et al., 2003; Lawrence et al., 2002; Matsumoto et al., 2002). While feeding irregularities may accompany mood disorders, so far, no link was demonstrated between Galp and depression/antidepressant actions.

The other transcript is alarin, a result of alternate splicing of Galp, which contains 25 amino acids (Santic et al., 2006, 2007). Alarin cannot bind to Gal1–3 and has no identified receptors so far (Lang et al., 2015), but is considered as a galanin system peptide because of its origin. Its distribution in the rodent brain contrasts that of the galanin-like peptide: it is abundant in cortical layers, the HC and the LC in mice among many other areas (Eberhard et al., 2012). Functionally alarin is involved in the regulation of feeding behavior and the modulation of the hypothalamus–pituitary–adrenal (HPA) axis (Fraley et al., 2012; Van Der Kolk et al., 2010). In addition, alarin has been implicated in depression by the recent studies of Wang et al., who proposed central roles for the modulation of hypothalamic hormones, the brain-derived neurotrophic factor and TrkB in its anti-depressive effects (Wang et al., 2014, 2015).

In rats, chronic fluoxetine (FLX) and paroxetine (PRX) treatments, which belong to the selective serotonin reuptake inhibitor (SSRI) class of antidepressants, elevated galanin expression in the DR (Lu et al., 2005; Rovin et al., 2012). In mice, chronic sertraline, another SSRI, or FLX treatments were able to induce similar changes in the HC region (Christiansen et al., 2011; Yamada et al., 2013). At the same time, chronic treatment with phenelzine (PLZ), a monoamino-oxidase inhibitor (MAOI) failed to cause any alterations in the gene expression of galanin and its receptors in the DR of rats (Rovin et al., 2012). The latter result suggests that the effects on galanin and Gal1–3 are not uniform among antidepressants and may be related to certain pharmacological properties. However, neither galanin, nor galanin receptor or Galp gene expression was studied after administration of a serotonin-noradrenaline or selective noradrenaline reuptake inhibitor antidepressant.

Venlafaxine (VLX) belongs to a group of drugs with an extended mechanism of action compared to SSRIs, that is they selectively inhibit both serotonin and noradrenaline reuptake (SNRIs). It has been used widely in clinical practice and been proven to be more effective than SSRIs in terms of economic costs, remission rates and earlier onset of antidepressant actions (Gex-Fabry et al., 2004; Smith et al., 2002). VLX has been shown to activate expression of a number of genes, for example such involved in neurotrophic signaling, glutamatergic transmission, neuroplasticity, synaptogenesis and cognitive processes (Tamasi et al., 2014). Interactions between noradrenergic neurotransmission and galanin signaling have been already discussed in the literature [for reviews see (Lu et al., 2007)], while those with the serotonergic system are described above. Thus, it is tempting to speculate that, if SSRIs are able to modulate galanin signaling, this may also be the case with SNRIs, in fact the effect could be enhanced.

In this report we present the results of a gene expression analysis of the galanin, Gal1, Gal2 and Gal3 and Galp genes from the DR, HC and FC regions of Dark Agouti (DaAg) rats following 3-week long chronic VLX treatment.

2. Materials and methods

The presented experiment was part of a large-scale microarray study, thus the methods used in this study are discussed in details in (Petschner et al., 2013; Tamasi et al., 2014), and here we only provide a brief description.

2.1. Animals and drugs

In this study altogether 20 male, DaAg rats (Harlan, Olac Ltd., Shaw's Farm, Blackthorn, Bicester, Oxon, UK, aged: 8 weeks, weighing 126.85 ± 4.22 g (mean ± S.E.M.) were used. The animal experiments and housing conditions were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC), the National Institutes of Health Principles of Laboratory Animal Care (NIH Publication 85-23, revised 1985) and special national laws (the Hungarian Governmental Regulation on animal studies, 31 December 1998 Act). The experiments were approved by the National Scientific Ethical Committee on Animal Experimentation and permitted by the Food Chain Safety and Animal Health Directorate of the Central Agricultural Office, Hungary (permission number: 22.1/3152/001/2007).

VLX was dissolved in 0.9% NaCl solution, and Alzet 2001 osmotic minipumps (Durect Corp., CA, USA) were filled with the solution.

2.2. Drug administration and experimental design

Alzet osmotic minipumps were inserted subcutaneously under the back skin of the rats in the VLX-groups while the control group underwent sham surgery. All surgery was performed under anesthesia with halothane. Due to the limited volume of the osmotic pumps, the sham surgery and minipump insertion had to be repeated every week for 3 weeks. The pumps delivered 40 mg/kg VLX each day, while food and water were available ad libitum. During surgical procedures one animal died, thus, altogether 19 animals were used in the experiments.

2.3. RNA extraction and sample preparation

Three weeks after the first osmotic minipump insertion rats were killed quickly by decapitation. The brains were removed, approximately 2 mm thick coronal sections were cut and the HC, FC and DR regions were dissected according to the Atlas of Paxinos and Watson (Paxinos and Watson, 1986) as follows: dorsal HC: from bregma −2.5 mm to −4.5 mm; FC: from bregma +1.7 to −0.3 mm; DR: from bregma −7 mm to −8 mm, respectively, and stored at −80 °C. The samples were homogenized with 1 ml TRIzol reagent and RNA was isolated. The pellets were dissolved in 20 μl diethylpyrocarbonate treated-dH2O (DEPC-dH2O) and solutions stored at −80 °C. The optical density (OD) ratios were determined for all samples for quality check and randomly repeated to evaluate the reliability of the measurements (no significant difference was observed, data not shown). Thereafter two-two randomly selected samples from the same treatment groups and region with the best OD ratios were pooled, resulting in altogether four pooled samples per treatment and region. From VLX treated and vehicle treated pools microarray experiments were performed by

Service XS (Leiden, Netherlands) on Illumina platforms (RatRef-12 v1 Beadarray Expression Chip, San Diego, CA, USA).

2.4. Data analysis

Raw microarray data were processed with beadarray (Dunning et al., 2007), preprocessCore (Bolstad) and puma (Pearson et al., 2009) Bioconductor (Gentleman et al., 2004) packages for R (R Core Team, 2012) as described in Alttoa et al. (2010). Briefly, backgroundCorrect method used in the beadarray package was set to "minimum", and "log = TRUE; n = 10" variables were used for createbeadsummaryData method. The normalization method was quantile normalization in the preprocessCore package. PumaComb, pumaDE, and write.rslts functions with default settings were used. Changes were considered statistically significant when the minimum probability of positive log ratio (MinPplr), a Bayesian-based value of significance, was below 0.001. This is a necessary and usual restriction to avoid false positive results in microarray experiments.

Data supporting the current results have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE47541 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE47541).

2.5. PCR validation

We have validated altogether 19 RNA products from the original pooled samples with real-time quantitative polymerase chain reaction (qPCR) on a Fluidigm GEx array (San Francisco, CA, USA) using Taqman Gene Expression assays for the appropriate RNAs obtained from Applied Biosystems (Carlsbad, CA, USA). The validation experiment was performed by Service XS (Leiden, Netherlands). Upon arrival of the normalized results, manually written R scripts using the cor.test function with default settings were used for the comparison between microarray and qPCR data. The Pearson correlation coefficients were 0.631 and 0.656 for the 200 ng and 500 ng samples, respectively, with p-values 5.3×10^{-14} and 2.2×10^{-15}.

2.6. Linear models

To statistically further validate the results on the galanin system's gene expression, linear models were fit on the normalized and background corrected microarray data using LIMMA (Smyth, 2005), a Bioconductor (Gentleman et al., 2004) package for R (R Core Team, 2012). During the analysis *lmFit* and *eBayes* functions with default settings were used. To obtain adjusted p-values the *topTable* function with the false discovery ratio (Benjamini and Hochberg, 1995) correction was used, a more permissive and accepted methodology for the reduction of false positive results than the stringent Bonferroni correction (Petschner et al., 2015).

3. Results and discussion

The antidepressant VLX was unable to alter the expression of galanin, Gal1, Gal2, Gal3 and Galp genes in the DR, HC and FC of DaAg rats (Table 1.).

Table 1/A. shows the minimum probability ratios for the galanin (Gal), galanin receptor 1 (Gal1), galanin receptor 2 (Gal2), galanin receptor 3 (Gal3) and galanin-like peptide precursor (Galp) genes in the examined brain regions following 40 mg/kg/die VLX treatment for 3 weeks (administered via osmotic minipumps implanted under the back skin) in Dark Agouti rats. Based on the minimum probability of positive log ratio (minPplr) method, only the Gal2 gene showed some alterations, however, due to all the genes represented on a single microarray plate, to avoid false positive results only values below 0.001 could have been considered significant. Table 1/B. shows the respective fold change values of the Gal, Gal1, Gal2, Gal3 and Galp genes in the different brain regions. Table 1/C. represents the false discovery ratio adjusted p-values of the VLX treated rats compared to the control animals using linear models, a basically different mathematical model than the minPplr method. The calculations were performed to provide further statistical support of the conclusions drawn in the paper. Fold change values are *per definitionem* the same for both methods.

Table 1
Galanin genes in different brain regions following a 3-week long VLX treatment of Dark Agouti rats.

A)

Minimum probability of positive log ratio values in different brain regions	Gal	Gal1	Gal2	Gal3	Galp
Brain regions					
Frontal cortex	0.397	0.225	0.351	0.450	0.395
Hippocampus	0.425	0.249	0.332	0.427	0.471
Dorsal raphe	0.333	0.327	0.017	0.466	0.429

B)

Fold change values in different brain regions	Gal	Gal1	Gal2	Gal3	Galp
Brain regions					
Frontal cortex	0.05	0.08	−0.06	0.10	−0.13
Hippocampus	−0.07	0.06	0.06	0.09	0.04
Dorsal raphe	0.12	−0.02	−0.22	0.04	−0.04

C)

The adjusted p-values in different brain regions after linear model fit	Gal	Gal1	Gal2	Gal3	Galp
Brain regions					
Frontal cortex	0.76	0.83	0.94	0.80	0.76
Hippocampus	0.99	0.99	0.99	0.99	0.99
Dorsal raphe	0.91	0.94	0.70	0.91	0.94

Table 2. shows the average, background corrected and normalized expression intensities for galanin system genes in the control animals. Since VLX caused no significant alterations in the level of these mRNAs a table containing those data is not included.

Several studies have explored the effects of various SSRIs and other drugs on the galanin system in different rodent experimental models. In the HC, Yamada and coworkers treated mice 4-week long with the SSRI sertraline and examined galanin in the dorsal and ventral dentate gyrus of mice (Yamada et al., 2013), where galanin was upregulated in the ventral but not in the dorsal dentate gyrus. Christiansen et al. combined a 14-day long FLX treatment with chronic restraint stress and found significantly elevated galanin expression in the dorsal HC, when compared with both stress-exposed and -unexposed rats without pharmacological intervention (Christiansen et al., 2011). Similarly, in the DR, Lu and coworkers have shown that chronic FLX could elevate galanin mRNA levels and Gal2-binding sites in the DR of rats (Lu et al., 2005). Rovin et al. studied chronic treatment (14 days) of Sprague–Dawley rats with the SSRI paroxetine and recorded, among others, elevated galanin levels in the DR; however, a 14-day long treatment with PLZ via osmotic minipumps failed to alter the expression of galanin (Rovin et al., 2012). PLZ, besides elevating 5-HT levels, also causes an increase in the level of NA and dopamine through inhibition of monoamine oxidases (Christmas et al., 1972) and influences so called trace amines, like 2-phenlyethylamine, tryptamine, octopamine, and tyramine (Baker et al., 1992).

Table 2
Average expression values of the galanin genes in the control animals.

Average expression values in different brain regions in the control group	Gal	Gal1	Gal2	Gal3	Galp
Brain regions					
Frontal cortex	141.70	88.76	152.30	107.89	137.41
Hippocampus	153.36	89.25	149.43	109.10	126.08
Dorsal raphe	436.21	89.74	233.35	110.94	120.90

These findings contrast our data showing lack of effect of chronic VLX, the "flagship" of SNRI antidepressants, on the expression of the galanin and its receptors' genes in the FC, HC and DR of normal DaAg rats. This is somewhat surprising, since VLX, like the above mentioned FLX and sertraline, potently inhibits the reuptake of 5-HT. One possible explanation is the fact that VLX also inhibits reuptake of NA causing an increase in extracellular concentration of this catecholamine. This hypothesis is supported by the fact that the MAO inhibitor PLZ, which also causes a parallel increase in 5-HT and NA, failed to cause any change in the expression of galanin and Gal1–3 in rats, indeed in four brain regions including DR (Rovin et al., 2012).

There is no direct evidence whether the increase of galanin gene expression by SSRIs increases or decreases their antidepressant properties. As discussed earlier, stimulation of Gal2 may cause antidepressant-like, stimulations of Gal1 and Gal3 receptors a pro-depressive effect. Our results may shed some light on this question. VLX as an antidepressant is at least as potent as the SSRIs, but without any effect on the galanin system. Thus, it is unlikely that the increased expression of galanin caused by the SSRIs is an important mediator of their antidepressant effects.

To what extent changes in galanin gene expression by SSRIs are responsible for antidepressant effect remains to be further analyzed in detail. However, as discussed earlier, stimulation of Gal2 may cause an antidepressant-like effect. If so, Lu et al.'s demonstration of FLX-induced increases in both galanin mRNA and, likely, Gal2 receptors (Lu et al., 2005), could result in a robust antidepressive effect. However, elevated galanin expression observed after chronic SSRI treatments may not necessarily represent beneficial changes in antidepressant properties. As earlier mentioned, stimulation of Gal2 may mediate antidepressant-like, whereas stimulation of Gal1 and Gal3 receptor a pro-depressive effect. The Gal1 receptor agonist, M617 augmented immobility time in the forced swimming test (Lundstrom et al., 2005), while Gal3 antagonists were able to reduce anxiety- and depression-like behavior in various models (Barr et al., 2006; Swanson et al., 2005). Gal3 KO mice exhibited an anxiety-like phenotype (Brunner et al., 2014), and decreased motivation elicited in mice by chronic pain requires a Gal1-triggered mechanism in the nucleus accumbens (Schwartz et al., 2014). At the same time, a Gal2 (and to a lesser extent Gal3) receptor agonist, AR-M1896, showed antidepressant properties (Kuteeva et al., 2008b). In accordance, i.c.v. administration of galanin on its own resulted in elevated immobility times in the forced swimming test in Sprague–Dawley rats, suggesting that elevated galanin levels may only have antidepressant properties when accompanied by decreased Gal1 and Gal3, or elevated Gal2 receptor expression or binding sites (Kuteeva et al., 2008b; Lu et al., 2005). This indicates that mood behavior may be influenced by a balance between different subtypes of activated galanin receptors.

Most of the studies using antidepressant treatments did not examine receptor levels to predict more exact consequences of the elevated galanin mRNA levels. Although, upregulated within the DR after FLX treatment (Lu et al., 2005), Gal2 receptors may also be of importance within the hippocampal formation. Thus, among the three galanin receptors, a robust expression of Gal2s has been demonstrated in the granule cells in the dentate gyrus (Burazin et al., 2000; O'Donnell et al., 1999). However, only one study has evaluated Gal2 levels from the HC following chronic sertraline treatment and reported elevated galanin, but unaltered Gal2 mRNA levels (Yamada et al., 2013).

We simultaneously examined the effects of an SNRI on the expression of galanin itself and its three receptors in the HC, DR and FC, showing a total lack of changes. Previous results from similar type of studies show that changes in galanin gene expression after chronic SSRI are difficult to interpret—are increases or decreases related to an antidepressant effect? Our study is unique in that VLX targets both the 5-HT and NA transporter, thus increasing extracellular levels of both monoamines. One conclusion is that the mechanism of antidepressant action of VLX likely does not include an effect via the galanin system. Since VLX is at least as potent as the SSRIs in treatment of depression, the significance of increased expression of galanin caused by the SSRIs remains to be further examined. It may be noted that the three monoamine systems, 5-HT, NA and dopamine are all connected. For example, it is known that the enhancement of 5-HT signaling after SSRIs will dampen the activity of the NA (and dopamine) neurons. Also the effects on certain monoamine receptors are different: SSRIs downregulate somato-dendritic 5-HT1A and nerve terminal 5-HT1B autoreceptors, SNRIs (and MAOIs) only the somato-dendritic ones [see e.g. (Blier and El Mansari, 2013)]. Differences like these may underlie the different results obtained with SSRIs versus SNRIs.

Another important potential target of VLX effects could have been the Galp gene. Galp encodes two proteins, galanin-like peptide and alarin. Since Galp's expression is limited to specific neurons in the hypothalamus (Jureus et al., 2000, 2001), our results may very well reflect alarin expression in DaAg rats. In support of the previous notion, Eberhard et al. demonstrated abundant alarin mRNA levels in the HC and FC of mice (Eberhard et al., 2012), similar to our results in the current experiment (see. Table 2.). The expression values, since microarray experiments are normalized both within and between arrays, may provide some rough insight in the relative expression of galanin system's transcripts within these regions. Our data show Galp having a higher signal than Gal1 or Gal3 within all three examined brain regions, suggesting that alarin may play important roles in these areas in rats. Please note, however, that the dissected brain regions are not well-characterized and do not allow us to draw explicit conclusions. Moreover, microarray experiments are not primarily designed to evaluate absolute expressions of differential transcripts due to the vast number of possible normalization and background correction methodologies.

Recently, Wang et al. demonstrated that i.c.v. administered alarin shows potential anti-depressive effects, accompanied by an upregulation of brain-derived neurotrophic factor in the prefrontal cortex and HC of depression-like mouse model (Wang et al., 2014). In addition, alarin also substantially influenced the levels of key hormones of the HPA axis in the same experiment, and more recent results suggest an involvement of TrkB and pro- and anti-inflammatory cytokines in the anti-depressive effects of alarin (Wang et al., 2015; Zhuang et al., 2016). While SSRIs may or may not include the modulation of alarin in their therapeutic efficacy, the lack of effects of the antidepressant VLX emphasizes, similarly to galanin and its receptors, alarin as potential target for future therapeutic agents.

4. Limitations and potential further studies

Finally, we have to raise attention to the limitations of the current study. First, we have to note that mRNA levels do not necessarily reflect appropriate protein levels; translation may occur at different degrees, or not at all. Second, dissected brain regions may be too large, and focusing on the role of subregions using laser dissection and/or in situ hybridization may reveal interesting results. For example sertraline-induced expression differences in the galanin gene are known to exist along the ventral–dorsal axis in the HC (Yamada et al., 2013). Third, multiple hypothesis testing may result in a significant amount of false positive results. To avoid such bias we decreased significance criterion to 0.001 for the minPplr method and used the false discovery ratio to obtain adjusted p-values when fitting linear models. Fourth, the effect of VLX should be studied in depression models, e.g. strong stress. Fifth, species/strain differences in the expression of the galanin system are known to exist (Le Maitre et al., 2013). For example, galanin is expressed in the 5-HT neurons in the rat DR, but not in mouse and human. Thus, comparisons between species with regard to antidepressant effects of various types of monoamine reuptake inhibitors should be made with caution. In addition, the expression of Galp observed in our study within the DR may reflect strain differences, since Eberhard et al. could not demonstrate similar expression levels in mice (Eberhard et al., 2012). Sixth, we have not directly validated the individual genes presented in the current

paper. However, we have validated the experimental methodology through other genes which resulted in overall more than 220 data points comparing the microarray and qRT-PCR data, providing good correlations (in the field of microarrays) and highly significant p-values. Furthermore, our statistical calculations were compared to the results of another accepted method in the field to reduce the chance of statistical errors and further support our conclusions.

5. Conclusions

We show that chronic VLX treatment fails to alter the gene expression of galanin, Gal1, Gal2, Gal3 and Galp in the DR, FC and HC of normal rats. These data suggest that the antidepressant therapeutic effect of serotonin-noradrenaline reuptake inhibitors is not mediated by the effects on galanin, its receptors or alarin. Thus, the galanin receptors and alarin remain promising therapeutic targets for about 40–50% of depressed patients, who are therapy resistant for the currently used antidepressants.

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References

Alttoa, A., Koiv, K., Hinsley, T.A., Brass, A., Harro, J., 2010. Differential gene expression in a rat model of depression based on persistent differences in exploratory activity. Eur. Neuropsychopharmacol. 20, 288–300.

Baker, G.B., Coutts, R.T., McKenna, K.F., Sherry-McKenna, R.L., 1992. Insights into the mechanisms of action of the MAO inhibitors phenelzine and tranylcypromine: a review. J. Psychiatry Neurosci. 17, 206–214.

Barr, A.M., Kinney, J.W., Hill, M.N., Lu, X., Biros, S., Rebek Jr., J., Bartfai, T., 2006. A novel, systemically active, selective galanin receptor type-3 ligand exhibits antidepressant-like activity in preclinical tests. Neurosci. Lett. 405, 111–115.

Barreda-Gomez, G., Giralt, M.T., Pazos, A., Rodriguez-Puertas, R., 2014. Galanin activated Gi/o-proteins in human and rat central nervous systems. Neuropeptides 48, 295–304.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Blier, P., El Mansari, M., 2013. Serotonin and beyond: therapeutics for major depression. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 368, 20120536.

Bolstad, B.M., preprocessCore: a collection of pre-processing functions. R Package Version 1.20.0.

Branchek, T.A., Smith, K.E., Gerald, C., Walker, M.W., 2000. Galanin receptor subtypes. Trends Pharmacol. Sci. 21, 109–117.

Brewer, A., Echevarria, D.J., Langel, U., Robinson, J.K., 2005. Assessment of new functional roles for galanin in the CNS. Neuropeptides 39, 323–326.

Brunner, S.M., Farzi, A., Locker, F., Holub, B.S., Drexel, M., Reichmann, F., Lang, A.A., Mayr, J.A., Vilches, J.J., Navarro, X., Lang, R., Sperk, G., Holzer, P., Kofler, B., 2014. GAL3 receptor KO mice exhibit an anxiety-like phenotype. Proc. Natl. Acad. Sci. U. S. A. 111, 7138–7143.

Burazin, T.C., Larm, J.A., Ryan, M.C., Gundlach, A.L., 2000. Galanin-R1 and -R2 receptor mRNA expression during the development of rat brain suggests differential subtype involvement in synaptic transmission and plasticity. Eur. J. Neurosci. 12, 2901–2917.

Christiansen, S.H., Olesen, M.V., Wortwein, G., Woldbye, D.P., 2011. Fluoxetine reverts chronic restraint stress-induced depression-like behaviour and increases neuropeptide Y and galanin expression in mice. Behav. Brain Res. 216, 585–591.

Christmas, A.J., Coulson, C.J., Maxwell, D.R., Riddell, D., 1972. A comparison of the pharmacological and biochemical properties of substrate-selective monoamine oxidase inhibitors. Br. J. Pharmacol. 45, 490–503.

Counts, S.E., He, B., Che, S., Ginsberg, S.D., Mufson, E.J., 2008. Galanin hyperinnervation upregulates choline acetyltransferase expression in cholinergic basal forebrain neurons in Alzheimer's disease. Neurodegener. Dis. 5, 228–231.

Dunning, M.J., Smith, M.L., Ritchie, M.E., Tavare, S., 2007. beadarray: R classes and methods for Illumina bead-based data. Bioinformatics 23, 2183–2184.

Eberhard, N., Mayer, C., Santic, R., Navio, R.P., Wagner, A., Bauer, H.C., Sperk, G., Boehm, U., Kofler, B., 2012. Distribution of alarin immunoreactivity in the mouse brain. J. Mol. Neurosci. 46, 18–32.

Edgar, R., Domrachev, M., Lash, A.E., 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30, 207–210.

Elvander, E., Schott, P.A., Sandin, J., Bjelke, B., Kehr, J., Yoshitake, T., Ogren, S.O., 2004. Intraseptal muscarinic ligands and galanin: influence on hippocampal acetylcholine and cognition. Neuroscience 126, 541–557.

Fraley, G.S., Leathley, E., Lundy, N., Chheng, E., King, I., Kofler, B., 2012. Effects of alarin on food intake, body weight and luteinizing hormone secretion in male mice. Neuropeptides 46, 99–104.

Fuxe, K., Jansson, A., Diaz-Cabiale, Z., Andersson, A., Tinner, B., Finnman, U.B., Misane, I., Razani, H., Wang, F.H., Agnati, L.F., Ogren, S.O., 1998. Galanin modulates 5-hydroxytryptamine functions. Focus on galanin and galanin fragment/5-hydroxytryptamine1A receptor interactions in the brain. Ann. N. Y. Acad. Sci. 863, 274–290.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J.Y., Zhang, J., 2004. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5, R80.

Gex-Fabry, M., Balant-Gorgia, A.E., Balant, L.P., Rudaz, S., Veuthey, J.L., Bertschy, G., 2004. Time course of clinical response to venlafaxine: relevance of plasma level and chirality. Eur. J. Clin. Pharmacol. 59, 883–891.

Han, D., Wang, E.C., 2005. Remission from depression: a review of venlafaxine clinical and economic evidence. PharmacoEconomics 23, 567–581.

Hansen, K.R., Krasnow, S.M., Nolan, M.A., Fraley, G.S., Baumgartner, J.W., Clifton, D.K., Steiner, R.A., 2003. Activation of the sympathetic nervous system by galanin-like peptide—a possible link between leptin and metabolism. Endocrinology 144, 4709–4717.

Holets, V.R., Hokfelt, T., Rokaeus, A., Terenius, L., Goldstein, M., 1988. Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. Neuroscience 24, 893–906.

Juhasz, G., Hullam, G., Eszlari, N., Gonda, X., Antal, P., Anderson, I.M., Hokfelt, T.G., Deakin, J.F., Bagdy, G., 2014. Brain galanin system genes interact with life stresses in depression-related phenotypes. Proc. Natl. Acad. Sci. U. S. A. 111, E1666–E1673.

Jureus, A., Cunningham, M.J., Li, D., Johnson, L.L., Krasnow, S.M., Teklemichael, D.N., Clifton, D.K., Steiner, R.A., 2001. Distribution and regulation of galanin-like peptide (GALP) in the hypothalamus of the mouse. Endocrinology 142, 5140–5144.

Jureus, A., Cunningham, M.J., McClain, M.E., Clifton, D.K., Steiner, R.A., 2000. Galanin-like peptide (GALP) is a target for regulation by leptin in the hypothalamus of the rat. Endocrinology 141, 2703–2706.

Kessler, R.C., Akiskal, H.S., Ames, M., Birnbaum, H., Greenberg, P., Hirschfeld, R.M., Jin, R., Merikangas, K.R., Simon, G.E., Wang, P.S., 2006. Prevalence and effects of mood disorders on work performance in a nationally representative sample of U.S. workers. Am. J. Psychiatry 163, 1561–1568.

Kohler, C., Persson, A., Melander, T., Theodorsson, E., Sedvall, G., Hokfelt, T., 1989. Distribution of galanin-binding sites in the monkey and human telencephalon: preliminary observations. Exp. Brain Res. 75, 375–380.

Kordower, J.H., Le, H.K., Mufson, E.J., 1992. Galanin immunoreactivity in the primate central nervous system. J. Comp. Neurol. 319, 479–500.

Kovac, S., Walker, M.C., 2013. Neuropeptides in epilepsy. Neuropeptides 47, 467–475.

Krasnow, S.M., Hohmann, J.G., Gragerov, A., Clifton, D.K., Steiner, R.A., 2004. Analysis of the contribution of galanin receptors 1 and 2 to the central actions of galanin-like peptide. Neuroendocrinology 79, 268–277.

Kuteeva, E., Hokfelt, T., Wardi, T., Ogren, S.O., 2008a. Galanin, galanin receptor subtypes and depression-like behaviour. Cell. Mol. Life Sci. 65, 1854–1863.

Kuteeva, E., Wardi, T., Lundstrom, L., Sollenberg, U., Langel, U., Hokfelt, T., Ogren, S.O., 2008b. Differential role of galanin receptors in the regulation of depression-like behavior and monoamine/stress-related genes at the cell body level. Neuropsychopharmacology 33, 2573–2585.

Lang, R., Berger, A., Santic, R., Geisberger, R., Hermann, A., Herzog, H., Kofler, B., 2005. Pharmacological and functional characterization of galanin-like peptide fragments as potent galanin receptor agonists. Neuropeptides 39, 179–184.

Lang, R., Gundlach, A.L., Holmes, F.E., Hobson, S.A., Wynick, D., Hokfelt, T., Kofler, B., 2015. Physiology, signaling, and pharmacology of galanin peptides and receptors: three decades of emerging diversity. Pharmacol. Rev. 67, 118–175.

Lawrence, C.B., Baudoin, F.M., Luckman, S.M., 2002. Centrally administered galanin-like peptide modifies food intake in the rat: a comparison with galanin. J. Neuroendocrinol. 14, 853–860.

Le Maitre, E., Barde, S.S., Palkovits, M., Diaz-Heijtz, R., Hokfelt, T.G., 2013. Distinct features of neurotransmitter systems in the human brain with focus on the galanin system in locus coeruleus and dorsal raphe. Proc. Natl. Acad. Sci. U. S. A. 110, E536–E545.

Lerner, J.T., Sankar, R., Mazarati, A.M., 2008. Galanin and epilepsy. Cell. Mol. Life Sci. 65, 1864–1871.

Lu, X., Barr, A.M., Kinney, J.W., Sanna, P., Conti, B., Behrens, M.M., Bartfai, T., 2005. A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus. Proc. Natl. Acad. Sci. U. S. A. 102, 874–879.

Lu, X., Sharkey, L., Bartfai, T., 2007. The brain galanin receptors: targets for novel antidepressant drugs. CNS Neurol. Disord. Drug Targets 6, 183–192.

Lundstrom, L., Elmquist, A., Bartfai, T., Langel, U., 2005. Galanin and its receptors in neurological disorders. Neruomol. Med. 7, 157–180.
Machado-Vieira, R., Salvadore, G., Luckenbaugh, D.A., Manji, H.K., Zarate Jr., C.A., 2008. Rapid onset of antidepressant action: a new paradigm in the research and treatment of major depressive disorder. J. Clin. Psychiatry 69, 946–958.
Matsumoto, Y., Watanabe, T., Adachi, Y., Itoh, T., Ohtaki, T., Onda, H., Kurokawa, T., Nishimura, O., Fujino, M., 2002. Galanin-like peptide stimulates food intake in the rat. Neurosci. Lett. 322, 67–69.
Melander, T., Hokfelt, T., Rokaeus, A., 1986. Distribution of galaninlike immunoreactivity in the rat central nervous system. J. Comp. Neurol. 248, 475–517.
Mennicken, F., Hoffert, C., Pelletier, M., Ahmad, S., O'Donnell, D., 2002. Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. J. Chem. Neuroanat. 24, 257–268.
Murck, H., Held, K., Ziegenbein, M., Kunzel, H., Holsboer, F., Steiger, A., 2004. Intravenous administration of the neuropeptide galanin has fast antidepressant efficacy and affects the sleep EEG. Psychoneuroendocrinology 29, 1205–1211.
O'Donnell, D., Ahmad, S., Wahlestedt, C., Walker, P., 1999. Expression of the novel galanin receptor subtype GALR2 in the adult rat CNS: distinct distribution from GALR1. J. Comp. Neurol. 409, 469–481.
O'Donnell, D., Mennicken, F., Hoffert, C., Hubatsch, D., Pelletier, M., Walker, P., Ahmad, S., 2002. Localization of galanin receptor subtypes in the rat CNS. Handbook of Chemical Neuroanatomy, Peptide Receptors, pp. 195–244.
Ohtaki, T., Kumano, S., Ishibashi, Y., Ogi, K., Matsui, H., Harada, M., Kitada, C., Kurokawa, T., Onda, H., Fujino, M., 1999. Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. J. Biol. Chem. 274, 37041–37045.
O'Leary, O.F., Dinan, T.G., Cryan, J.F., 2015. Faster, better, stronger: towards new antidepressant therapeutic strategies. Eur. J. Pharmacol. 753, 32–50.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. second ed. Academic Press, Sydney; Orlando.
Pearson, R.D., Liu, X., Sanguinetti, G., Milo, M., Lawrence, N.D., Rattray, M., 2009. puma: a Bioconductor package for propagating uncertainty in microarray analysis. BMC Bioinf. 10, 211.
Petschner, P., Bagdy, G., Tothfalusi, L., 2015. [The problem of small "n" and big "P" in neuropsycho-pharmacology, or how to keep the rate of false discoveries under control]. Neuropsychopharmacologia Hungarica: a Magyar Pszichofarmakologiai Egyesulet Lapja = official journal of the Hungarian Association of Psychopharmacology 17, 23–30.
Petschner, P., Tamasi, V., Adori, C., Kirilly, E., Ando, R.D., Tothfalusi, L., Bagdy, G., 2013. Gene expression analysis indicates CB1 receptor upregulation in the hippocampus and neurotoxic effects in the frontal cortex 3 weeks after single-dose MDMA administration in Dark Agouti rats. BMC Genomics 14, 930.
Picciotto, M.R., 2010. Galanin and addiction. EXS 102, 195–208.
Pieribone, V.A., Xu, Z.Q., Zhang, X., Hokfelt, T., 1998. Electrophysiologic effects of galanin on neurons of the central nervous system. Ann. N. Y. Acad. Sci. 863, 264–273.
Pigott, H.E., Leventhal, A.M., Alter, G.S., Boren, J.J., 2010. Efficacy and effectiveness of antidepressants: current status of research. Psychother. Psychosom. 79, 267–279.
R Core Team, 2012. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.
Rovin, M.L., Boss-Williams, K.A., Alisch, R.S., Ritchie, J.C., Weinshenker, D., West, C.H., Weiss, J.M., 2012. Influence of chronic administration of antidepressant drugs on mRNA for galanin, galanin receptors, and tyrosine hydroxylase in catecholaminergic and serotonergic cell-body regions in rat brain. Neuropeptides 46, 81–91.
Saar, I., Lahe, J., Langel, K., Runesson, J., Webling, K., Jarv, J., Rytkonen, J., Narvanen, A., Bartfai, T., Kurrikoff, K., Langel, U., 2013a. Novel systemically active galanin receptor 2 ligands in depression-like behavior. J. Neurochem. 127, 114–123.
Saar, I., Runesson, J., Jarv, J., Kurrikoff, K., Langel, U., 2013b. Novel galanin receptor subtype specific ligand in depression like behavior. Neurochem. Res. 38, 398–404.
Santic, R., Fenninger, K., Graf, K., Schneider, R., Hauser-Kronberger, C., Schilling, F.H., Kogner, P., Ratschek, M., Jones, N., Sperl, W., Kofler, B., 2006. Gangliocytes in neuroblastic tumors express alarin, a novel peptide derived by differential splicing of the galanin-like peptide gene. J. Mol. Neurosci. 29, 145–152.
Santic, R., Schmidhuber, S.M., Lang, R., Rauch, I., Voglas, E., Eberhard, N., Bauer, J.W., Brain, S.D., Kofler, B., 2007. Alarin is a vasoactive peptide. Proc. Natl. Acad. Sci. U. S. A. 104, 10217–10222.
Schwartz, N., Temkin, P., Jurado, S., Lim, B.K., Heifets, B.D., Polepalli, J.S., Malenka, R.C., 2014. Chronic pain. Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. Science 345, 535–542.
Senut, M.C., Menetrey, D., Lamour, Y., 1989. Cholinergic and peptidergic projections from the medial septum and the nucleus of the diagonal band of broca to dorsal hippocampus, cingulate cortex and olfactory bulb: a combined wheatgerm agglutinin-apohorseradish peroxidase-gold immunohistochemical study. Neuroscience 30, 385–403.
Sharkey, L.M., Madamba, S.G., Siggins, G.R., Bartfai, T., 2008. Galanin alters GABAergic neurotransmission in the dorsal raphe nucleus. Neurochem. Res. 33, 285–291.
Skofitsch, G., Jacobowitz, D.M., 1985. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides 6, 509–546.
Skofitsch, G., Sills, M.A., Jacobowitz, D.M., 1986. Autoradiographic distribution of 125I-galanin binding sites in the rat central nervous system. Peptides 7, 1029–1042.
Smith, D., Dempster, C., Glanville, J., Freemantle, N., Anderson, I., 2002. Efficacy and tolerability of venlafaxine compared with selective serotonin reuptake inhibitors and other antidepressants: a meta-analysis. Br. J. Psychiatry J. Ment. Sci. 180, 396–404.
Smyth, G.K., 2005. limma: Linear Models for Microarray Data. In: Gentleman, R., Carey, V., Huber, W., Irizarry, R., Dudoit, S. (Eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Springer, New York, pp. 397–420.
Swanson, C.J., Blackburn, T.P., Zhang, X., Zheng, K., Xu, Z.Q., Hokfelt, T., Wolinsky, T.D., Konkel, M.J., Chen, H., Zhong, H., Walker, M.W., Craig, D.A., Gerald, C.P., Branchek, T.A., 2005. Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. Proc. Natl. Acad. Sci. U. S. A. 102, 17489–17494.
Tamasi, V., Petschner, P., Adori, C., Kirilly, E., Ando, R.D., Tothfalusi, L., Juhasz, G., Bagdy, G., 2014. Transcriptional evidence for the role of chronic venlafaxine treatment in neurotrophic signaling and neuroplasticity including also glutamatergic [corrected] - and insulin-mediated neuronal processes. PLoS One 9, e113662.
Tatemoto, K., Rokaeus, A., Jornvall, H., McDonald, T.J., Mutt, V., 1983. Galanin—a novel biologically active peptide from porcine intestine. FEBS Lett. 164, 124–128.
Thase, M.E., Haight, B.R., Richard, N., Rockett, C.B., Mitton, M., Modell, J.G., VanMeter, S., Harriett, A.E., Wang, Y., 2005. Remission rates following antidepressant therapy with bupropion or selective serotonin reuptake inhibitors: a meta-analysis of original data from 7 randomized controlled trials. J. Clin. Psychiatry 66, 974–981.
Trivedi, M.H., Rush, A.J., Wisniewski, S.R., Nierenberg, A.A., Warden, D., Ritz, L., Norquist, G., Howland, R.H., Lebowitz, B., McGrath, P.J., Shores-Wilson, K., Biggs, M.M., Balasubramani, G.K., Fava, M., Team, S.D.S., 2006. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am. J. Psychiatry 163, 28–40.
Van Der Kolk, N., Madison, F.N., Mohr, M., Eberhard, N., Kofler, B., Fraley, G.S., 2010. Alarin stimulates food intake in male rats and LH secretion in castrated male rats. Neuropeptides 44, 333–340.
Wang, M., Chen, Q., Li, M., Zhou, W., Ma, T., Wang, Y., Gu, S., 2014. Alarin-induced antidepressant-like effects and their relationship with hypothalamus–pituitary–adrenal axis activity and brain derived neurotrophic factor levels in mice. Peptides 56, 163–172.
Wang, M., Zhou, W., Zhou, X., Zhuang, F., Chen, Q., Li, M., Ma, T., Gu, S., 2015. Antidepressant-like effects of alarin produced by activation of TrkB receptor signaling pathways in chronic stress mice. Behav. Brain Res. 280, 128–140.
Weiss, J.M., Bonsall, R.W., Demetrikopoulos, M.K., Emery, M.S., West, C.H., 1998. Galanin: a significant role in depression? Ann. N. Y. Acad. Sci. 863, 364–382.
Wray, N.R., Pergadia, M.L., Blackwood, D.H., Penninx, B.W., Gordon, S.D., Nyholt, D.R., Ripke, S., MacIntyre, D.J., McGhee, K.A., Maclean, A.W., Smit, J.H., Hottenga, J.J., Willemsen, G., Middeldorp, C.M., de Geus, E.J., Lewis, C.M., McGuffin, P., Hickie, I.B., van den Oord, E.J., Liu, J.Z., Macgregor, S., McEvoy, B.P., Byrne, E.M., Medland, S.E., Statham, D.J., Henders, A.K., Heath, A.C., Montgomery, G.W., Martin, N.G., Boomsma, D.I., Madden, P.A., Sullivan, P.F., 2012. Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. Mol. Psychiatry 17, 36–48.
Xu, X.J., Hokfelt, T., Wiesenfeld-Hallin, Z., 2008. Galanin and spinal pain mechanisms: where do we stand in 2008? Cell. Mol. Life Sci. 65, 1813–1819.
Xu, Z.Q., Hokfelt, T., 1997. Expression of galanin and nitric oxide synthase in subpopulations of serotonin neurons of the rat dorsal raphe nucleus. J. Chem. Neuroanat. 13, 169–187.
Yamada, M., Makino, Y., Hashimoto, T., Sugiyama, A., Oka, J., Inagaki, M., Yamada, M., Saitoh, A., 2013. Induction of galanin after chronic sertraline treatment in mouse ventral dentate gyrus. Brain Res. 1516, 76–82.
Zhuang, F., Zhou, X., Gao, X., Lou, D., Bi, X., Qin, S., Sun, C., Ye, P., Wang, Y., Ma, T., Li, M., Gu, S., 2016. Cytokines and glucocorticoid receptors are associated with the antidepressant-like effect of alarin. Peptides.