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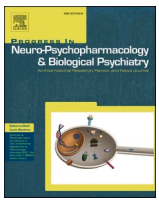

A new pharmacological strategy against treatment-resistant depression

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ABSTRACT

Major depressive disorder affects more than 50 million people in the world. However, 50% of patients don't respond to two or more drugs or psychotherapeutic treatments, named treatment-resistant depression (TRD). In this work, we propose a new augmentation treatment against TRD based on combining Fluoxetine (FLX) and the N-terminal fragment Galanin, GAL(1–15). In Wistar Kyoto (WKY) rats, akin to endogenous depression genetically, we evaluate GAL(1–15)'s impact on FLX-induced behaviours on tests measuring despair and anhedonia. We explored GALR2 involvement using the antagonist M871 and an in vivo model with siRNA 5-HT1A knockdown. Also, the 5-HT1AR was analyzed by autoradiography binding in several brain regions. We analyze the corticosterone levels and a dexamethasone-suppressed corticotropin-releasing hormone stimulation to study the HPA axis regulation. Our results shows that only the combination of FLX + GAL(1–15) induced antidepressant effects in the WKY animals in Behavioural tests related to despair. This combination also reduced corticosterone levels in the WKY animals and modulated the functional characteristics of the serotoninergic receptor 5-HT1A in the prefrontal cortex. These novel results suggest combining GAL(1–15) with FLX is a new potentiation strategy in TRD cases. It shows the innovative potential of the interactions between the galaninergic and serotonergic systems to find new strategies and drugs against resistant depression.

1. Introduction

Major depressive disorder (MDD), the most prevalent psychiatric disorder, is the leading cause of severe physical disability and suicide worldwide. According to estimates by the World Health Organization, in 2030, it will become the main cause of incapacity in the world (Chisholm et al., 2016).

In addition, the coronavirus disease 2019 (COVID-19) pandemic has created an environment in which many determinants of mental health are also affected. A recent study estimated an additional 53,2 million cases of MDD globally due to the COVID-19 pandemic ("Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic," 2021).

MDD is characterized by hopelessness, anhedonia, exacerbated guilt and painful physical symptoms (Artigas et al., 2018), often leading to suicidal tendencies (Kessler and Bromet, 2013). Medications for MDD primarily adjust serotonin (5-HT) and norepinephrine neurotransmission. The most common initial choice is selective 5-HT reuptake inhibitor antidepressants (SSRIs) due to their strong efficacy, tolerability, and treatment adherence. However, 50% of patients don't respond to two or more drugs or psychotherapeutic treatments, named treatment-resistant depression (TRD) (Fekadu et al., 2009; Trivedi and Daly, 2008). Thus, innovative solutions are imperative to provide effective, faster and prolonged relief of depressive symptoms in TRD patients.

The concept of TRD is not universally accepted, and several authors consider the term difficult-to-treat depression (DTD) to be more appropriate, taking a holistic approach and considering all treatment options available. DTD is defined as depression that continues to cause a significant burden despite usual treatment efforts and may provide a more clinically useful conceptualization of patients with TRD as it

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implies a search for the obstacles that prevent the achievement of a sustained symptom-free state with a return to premorbid function. When that ideal goal is unmet, the intervention changes to optimize symptom control, function, and quality of life over the long term. However, since the bibliography consulted uses the concept of TRD, we will continue with this for greater clarity and homogeneity (McAllister-Williams et al., 2020, 2021).

In this context, certain members of the Galanin (GAL) neuropeptide family are implicated in depression development (Fuxe et al., 2012; Fuxe et al., 2008), offering a promising chance to enhance antidepressant efficacy by combining them with these neuropeptides.

GAL is involved in various physiological processes and diseases in animal models, including mood regulation and depression (Díaz-Cabiale et al., 2010; Lang et al., 2007; Millón et al., 2014; Mitsukawa et al., 2010), through its three-GAL receptors (GALR) (Branchek et al., 2000). GALR1 and GALR3 activation is linked to depression-like behaviour, while GALR2 stimulation has anti-depressant-like effects (Bartfai et al., 2004; Kuteeva et al., 2008; Lang et al., 2007; Lu et al., 2005). Chronic use of the SSRI Fluoxetine (FLX) and electroconvulsive treatment raises GAL mRNA levels in the rat's dorsal raphe (DR). This increase is accompanied by higher GALR2 receptor binding sites in this monoaminergic nucleus, whereas GALR1 receptor binding sites remain unaffected.

The N-terminal fragment GAL(1–15) is also active in mood disorders (Millón et al., 2019; Millón et al., 2014); GAL(1–15) acting through GALR1-GALR2 heteroreceptor complexes, especially in the dorsal hippocampus and DR (Borroto-Escuela et al., 2014; Millón et al., 2014) results in depression and anxiogenic-like effects. Interestingly, GAL (1–15) enhanced the antidepressant effects induced by the 5-HT1AR agonist 8-OH-DPAT in the forced swimming test (FST) (Millón et al., 2016), the effect that was significantly stronger than the ones induced by GAL. This action's mechanism involved alterations in the binding characteristics and mRNA levels of 5-HT1AR in the dorsal hippocampus and DR.

Based on these findings, we analyzed the potential use of GAL(1–15) as a combined treatment with SSRIs. In rat studies, GAL(1–15) enhanced the antidepressant effects and reversed the memory impairment induced by FLX (Flores-Burgess et al., 2019; Flores-Burgess et al., 2017) being involved the 5-HT1AR in the hippocampus and prefrontal cortex (PFC), respectively (Flores-Burgess et al., 2019; Flores-Burgess et al., 2017). The interaction between GAL(1–15) and FLX was further investigated in a depression model using olfactory bulbectomy (OBX) rats (Flores-Burgess et al., 2022). GAL(1–15) strengthens FLX's antidepressant-like impact in despair and anhedonia tests. This process relies on 5-HT1AR in the hippocampus at the plasma membrane and transcriptional levels (Flores-Burgess et al., 2022). GAL(1–15) also enhances Escitalopram's impact on depressive behaviour tests in OBX rats, involving 5-HT1AR activation. This effect is inhibited when 5-HT1AR downregulation is induced using siRNA (García-Durán et al., 2021). Two networks are implicated in the effect of GAL(1–15) in Escitalopram activity: one involving lateral (LHb) and medial (mHb) habenula, dorsal raphe (DR) and ventral tegmental area (VTA), and the other including dentate gyrus (DG) and PFC (García-Durán et al., 2021).

While indicating a novel approach to depression treatment, further exploration of GAL(1–15) and FLX co-administration effects in TRD is necessary. To delve deeper, we have selected the Wistar-Kyoto (WKY) rat model, which is akin to endogenous depression genetically (Aleksandrova et al., 2019). This strain fulfils depression model criteria and mirrors monoamine, glutamate, and GABA system irregularities and HPA axis dysregulation seen in MDD patients. Standard antidepressants, especially SSRIs, don't elicit responses in these WKY rats, making them a strong TRD candidate (Aleksandrova et al., 2019).

To evaluate GAL(1–15)'s impact on FLX-induced behaviours in WKY rats, we examined its effects on tests measuring despair and anhedonia. We explored GALR2 involvement using the antagonist M871 and an in vivo model with siRNA 5-HT1A knockdown. We also investigated if GAL (1–15)'s effects on FLX were linked to 5-HT1AR, analyzing the 5-HT1AR binding in brain regions (DR, DG, CA1, ventral hippocampus and mPFC) of WKY rats treated with GAL(1–15) + FLX. Using Principal Component Analysis (PCA), we assessed the relationship between FST results (immobility, swimming) and Kd/Bmax autoradiographic data.

Moreover, we have also studied the corticosterone levels and a dexamethasone-suppressed corticotropin-releasing hormone stimulation (DEX/CRH) test to study the effects on the HPA axis regulation of the administration of GAL(1–15) + FLX in WKY animals. We have studied the mRNA expression levels of glucocorticoid (GR) and mineralocorticoid receptors (MR), 5-HT1AR, GALR1 and GALR2, BDNF and TrkB in the WKY rats treated with GAL(1–15) + FLX in different brain regions of interest.

2. Material and methods

Detailed descriptions on experimental design (Fig. 1), animals, administration of substances and drugs or technical and surgical procedures are available in the supplementary material.

3. Results

3.1. Behavioural effects

Before examining the behavioural effects of the FLX + GAL(1–15) combination in the WKY animals, we established the depressive-like behavioural profile of these animals to the tests employed in our research. We compared them to the outbred Wistar rat strain.

In the FST, WKY animals showed a significant statistical increase in the immobility time ($t_{18} = 3004$, $p = 0.0076$) and a decrease in the climbing ($t_{18} = 4927$, $p < 0.001$) compared with the Wistar animals (as shown in table S1). These findings were consistent with outcomes from the TST, a widely-used assessment of rodent despair behaviour. In the TST, WKY animals displayed an increased immobility time ($t_{10} = 2.864$, $p = 0.0168$) once again compared to Wistar rats (table S1).

Conversely, no disparities were observed between WKY animals and Wistar rats regarding preference for or consumption of sucrose in the SPT conducted in our laboratory (table S1).

The coadministration of GAL(1–15) and FLX induce an antidepressant-like effect in WKY rats in behavioural test. Involvement of GALR2 and 5HT1AR.

In the FST, only the WKY animal group administered with GAL (1–15)-FLX showed an antidepressant-like effect with a significant statistically decreased in immobility time ($F_{3,33} = 5.800$, $P = 0.0027$; Fisher's LSD post hoc: $P < 0.05$) (Fig. 2A) and an increase in the swimming time ($F_{3,33} = 5.159$, $P = 0.0049$; Fisher's LSD post hoc: $P < 0.05$) (Fig. 2B) compared with WKY control group. No difference was found in climbing time due to treatments.

As previously described, the doses and pattern of administration of FLX used in this study lacked any antidepressant-like effect in the FST.

We tested the role of GALR2 in the antidepressant-like effects induced by the GAL(1–15)-FLX combination using the antagonist M871. M871(3 nmol) blocked the reduction of the immobility time (Fisher's LSD post hoc: $P < 0.05$) (Fig. 2A) and the increase in swimming time (Fisher's LSD post hoc: $P < 0.05$) (Fig. 2B) found after the coadministration of GAL(1–15) and FLX in the FST.

GAL(1–15)(1 nmol) and M871(3 nmol) administered alone lacked an effect in the FST in WKY animals (Fig. 2C).

We also studied the role of 5HT1AR in this interaction employing siRNA 5HT1AR knockdown WKY animals. WKY rats icv injected with a single dose of siRNA 5HT1A reduced the immunoreactivity of 5-HT1AR in the DR ($t_{14} = 4665$, $p < 0.001$) 14 days after the injection (Fig. S1) as previously described (García-Durán et al., 2021).

siRNA 5HT1AR knockdown in WKY rats validates the involvement of 5-HT1AR in the effects induced by GAL(1–15). The decrease in 5-HT1AR was sufficient to block GAL(1–15) enhancement of the antidepressant-

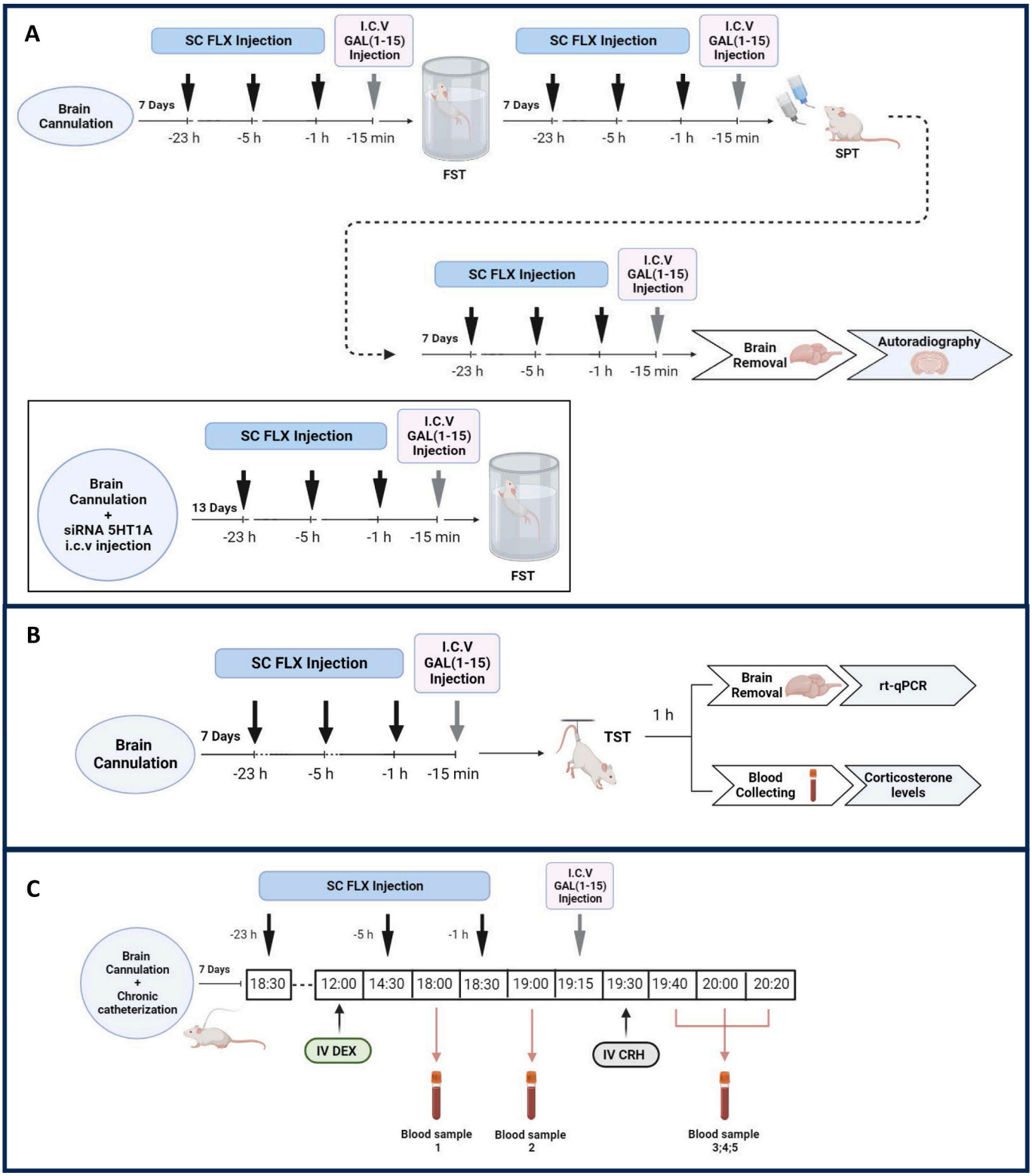
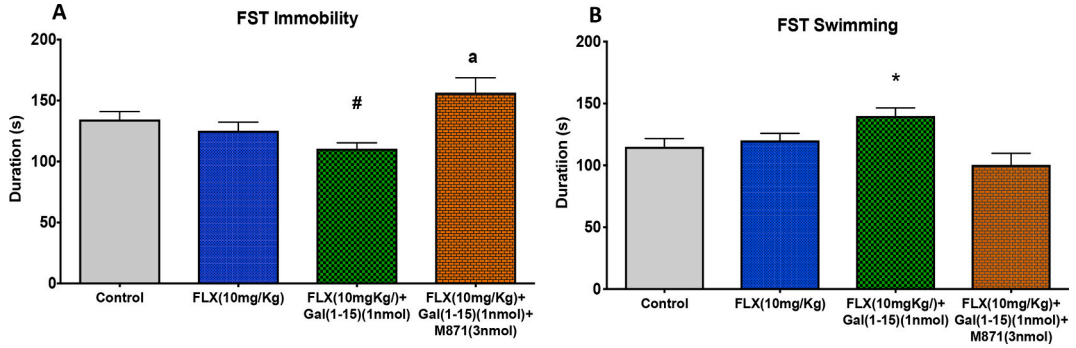


Fig. 1. Experimental schedule diagram. After brain cannulation, all animals had a recovery period before performing behavioural tests. A) The effects of different pharmacological treatments in the forced swimming test (FST) and the sucrose preference test (SPT) was evaluated in different groups of WKY rats. One week after the behavioural tests, we performed the autoradiography experiments. We also use a knockdown 5-HT1AR WKY animals to assess the pharmacological treatments in the FST. B) In other sets of animals, we evaluated the effects of different pharmacological treatments in the tail suspension test (TST). One hour after the TST, we collected the animals' blood and brain to perform the qPCR-rt experiments and determine the corticosterone blood levels. C) In the last set of animal, we evaluated the effects of the different pharmacological treatments on the corticosterone levels during the DEX/CRH test.



C

	Control	GAL(1-15)(1nmol)	M871(3nmol)
Immobility (s.)	140.6±7.9	133.2±25.9	161.9±17.8
Swimming (s.)	109.5±8.8	122.6±18.6	103.6±10.8

D

	Control	GAL(1-15)(1nmol)	FLX(10mg/kg)	FLX(10mg/kg)+ GAL(1-15)(1nmol)
Immobility (s.)	155±27.9	134.2±20.7	179±26	126.7±10.2
Swimming (s.)	77.7±21	91.3±14.6	59.1±21.8	94.6±7.5

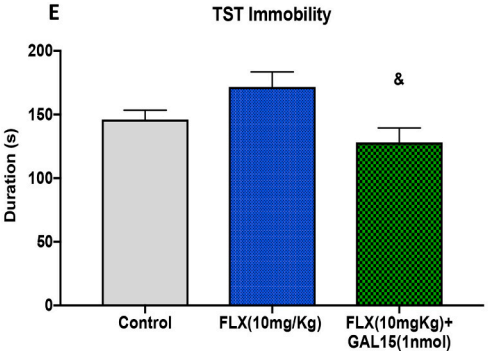


Fig. 2. Behavioural effects of the coadministration of FLX(10 mg/kg) alone or in combination with GAL(1–15)(1 nmol) or GAL(1–15) + M871(3 nmol) in the A) immobility time and B) swimming time in the FST. FLX was administered sc 23, 5 and 1 h before the tests, and aCSF, GAL(1–15) or GAL(1–15) + M871 was injected icv 15 min before the tests. Vertical bars represent the mean ± SEM ($n = 11$–6). *$p < 0.05$ versus rest of the groups, # $p < 0.05$ versus Control and FLX(10 mg/kg) + GAL(1–15)(1 nmol) + M871(3 nmol) groups; a $p < 0.05$ versus FLX(10 mg/kg) and FLX(10 mg/kg) + GAL(1–15)(1 nmol) groups. C) Effects of GAL(1–15)(1 nmol) or M871(3 nmol) administered icv alone 15 min before FST in WKY rats. No statistically significant differences were observed in the immobility or swimming time. D) Behavioural effects of the coadministration of FLX(10 mg/kg) alone or in combination with GAL(1–15)(1 nmol) in the Knock-down 5HT1A WKY rats in FST. No statistically significant differences were observed in the immobility or swimming time in this animal model. E) Behavioural effects of the coadministration of FLX(10 mg/kg) alone or in combination with GAL(1–15)(1 nmol) in the immobility time in the TST. & $p < 0.01$ versus FLX(10 mg/kg) group according to one way ANOVA followed by Fisher's least significance difference test.

like effects mediated by FLX in the FST (Fig. 2D). Thus, the coadministration of GAL(1–15) + FLX lacked effect on the immobility and swimming time in the FST (Fig. 2D).

The impact of the GAL(1–15)-FLX combination on WKY animals' behaviour was substantiated in the TST, where the coadministration of GAL(1–15) with FLX led to a reduction in immobility time compared to FLX alone ($F_{2,20} = 4.475$, $P = 0.0248$; Fisher's LSD post hoc: $P < 0.01$) (shown in Fig. 2E). Conversely, in the SPT, the GAL(1–15)-FLX combination lacked effects preference or sucrose intake in WKY animals (refer to Table S2).

3.2. Neurochemical effects

3.2.1. 5-HT1AR agonist radioligand binding. Saturation curve

In this study, we analyzed the effects on the [^{3}H]-8-OH-DPAT binding characteristics in the mPFC, DR, DG and CA1 of the dorsal hippocampus and ventral hippocampus of WKY rats treated with the combination GAL(1–15) + FLX. Table 1 describes the effects of the different treatments in the Kd or Bmax parameters in all the areas analyzed.

In the PFC, we observed a decrease in the Kd value as a result of administering FLX or GAL(1–15) + FLX ($F_{2,15} = 5.736$, $P < 0.0141$). Additionally, there was an elevation in the Bmax in this region ($F_{2,15} = 95.49$, $P < 0.001$). However, the group treated with GAL(1–15) + FLX exhibited a lesser increase in comparison to the FLX-treated WKY animals (Fisher's LSD post hoc: $P < 0.01$).

In DR, we observed an increase of the Bmax induced by the administration of FLX or GAL(1–15) + FLX ($F_{2,15} = 9.045$, $P = 0.0026$; Fisher's LSD post hoc: $P < 0.01$) without any changes observed in the Kd value.

Conversely, in the CA1 region of the dorsal hippocampus, both FLX and the combination of GAL(1–15) + FLX resulted in a decrease in Bmax ($F_{2,15} = 7.409$, $P = 0.0058$), with no observed changes in the Kd value. In the DG, it was only the GAL(1–15) + FLX combination that led to a decrease in Bmax ($F_{2,15} = 4.725$, $P = 0.0256$). Regarding the Kd value, the reduction was induced only by the FLX group ($F_{2,15} = 59.68$, $P < 0.001$).

Within the ventral hippocampus, both FLX and the combination of GAL(1–15) + FLX triggered a reduction in the Bmax ($F_{2,15} = 7.741$, $P = 0.0049$), along with a significant decrease in the Kd ($F_{2,15} = 115.3$, $P < 0.001$).

3.2.2. PCA

We conducted two PCAs to analyze the interrelationship between FST data (immobility and swimming time) and the Kd/Bmax autoradiographic parameters.

The first PCA included the FST measures and the autoradiographic Kd parameters as variables. Sample adequacy tests revealed that the data was suitable for PCA (Kaiser–Meyer–Olkin, KMO = 0.584; Bartlett's sphericity test: $X^2(21) = 43.165$, $P = 0.003$). Three independent components accounting for 78.8% of the total variance were extracted (Fig. 3A). Component 1 (variance explained ~35.6%) was representative of the FST performance and the Kd values in PFC, with low PC scores indicating less immobility and more swimming during the FST task and lower Kd values in PFC. Component 2 (variance explained ~28.5%) included the Kd of the DR, ventral hippocampus, and DG of the dorsal hippocampus with low PC scores indicating high Kd levels in hippocampal regions and low Kd values in the DR. Component 3 (variance explained ~14.7%) included the CA1 Kd (Fig. 3E). To determine

Table 1
Effects of the administration of FLX(10 mg/kg) alone or in combination with GAL(1–15)(1 nmol) on the binding characteristics of 5-HT1AR agonist [^{3}H]-8-OHDPAT in the medial Prefrontal cortex (PFC), Dorsal raphe (DR), CA1 and dentate gyrus (DG) of the dorsal hippocampus and ventral hippocampus (v-Hypo) of WKY rats. FLX was administered sc 23, 5 and 1.25 h before rats were euthanized. aCSF or GAL(1–15) was injected icv 30 min before rats were euthanized. Saturation experiments were performed with 10 concentrations of [^{3}H]-8-OHDPAT (0.26–10 nM) in different sections of each brain region. Non-specific binding was defined as the binding in the presence of 10 mM serotonin. Data are represented as mean ± SEM ($n = 6$) of Kd and Bmax values. *$p <$ 0.05, **$p < 0.01$ ***$p < 0.001$ compared versus Control groups, # $p < 0.001$ versus Control and FLX(10 mg) + GAL(1–15)(1 nmol) group, & $p < 0.001$ versus Control and FLX(10 mg) group according to one-way ANOVA followed by Fisher's least significance difference test.

		Kd (nM)	Bmax (fmol/mg prot)
PFC	Control	0,24 ± 0,03	1752 ± 48
	FLX(10 mg/Kg)	0,17 ± 0,01 **	2641 ± 54,5 #
	FLX(10 mg/Kg) + GAL(1–15)(1 nmol)	0,14 ± 0,01 *	2252 ± 31 &
DR	Control	0,53 ± 0,03	2737 ± 96
	FLX(10 mg/Kg)	0,71 ± 0,12	4100 ± 331 **
	FLX(10 mg/Kg) + GAL(1–15)(1 nmol)	0,61 ± 0,08	3990 ± 266 **
CA1	Control	0,6 ± 0,03	2842 ± 108
	FLX(10 mg/Kg)	0,51 ± 0,04	2494 ± 25 **
	FLX(10 mg/Kg) + GAL(1–15)(1 nmol)	0,5 ± 0,02	2613 ± 21 *
DG	Control	0,56 ± 0,04	2880 ± 74
	FLX(10 mg/Kg)	0,15 ± 0,01 #	2746 ± 32
	FLX(10 mg/Kg) + GAL(1–15)(1 nmol)	0,48 ± 0,02	2673 ± 24 **
v-Hyp	Control	0,78 ± 0,02	2755 ± 96
	FLX(10 mg/Kg)	0,39 ± 0,02***	2393 ± 93 **
	FLX(10 mg/Kg) + GAL(1–15)(1 nmol)	0,42 ± 0,36***	2350 ± 36 **

whether the experimental groups differed in the PC scores extracted in the analysis a one-way ANOVA was performed on each of the components. Regarding Component 1, we observed that there was a significant reduction in the PC scores in GAL(1–15) + FLX-treated animal group ($F_{2,15} = 6532$, $P = 0.0091$) (Fig. 3C), indicating that animals from this group showed lower despair behaviour together with lower Kd values in PFC. In Component 2, we found a reduction in the PC score in the FLX-treated animals ($F_{2,15} = 11{,}85$, $P = 0.008$), which indicates that rats from this group showed higher Kd values in the hippocampal regions along with lower Kd values in DR (Fig. 3D). In Component 3, no significant differences were observed between groups in PC scores between groups (Fig. 3E).

A second PCA was conducted, including the FST measures and the autoradiographic Bmax parameters. Again, sample adequacy tests revealed that the data was suitable for PCA (KMO = 0.544; Bartlett's sphericity test: $X^2(21) = 60.154$, $P < 0.001$). The analysis revealed two independent components accounting for 70% of the total variance (Fig. 4F). Component 1 (variance explained ~44.4%) was representative of Bmax values in all brain areas analyzed, with high PC scores indicating high Bmax levels in hippocampal regions and low Bmax values in the DR and PFC. Component 2 (variance explained ~25.6%) was representative of the FST performance, with low PC scores indicating less immobility and more swimming during the task. The one-way ANOVA analysis of PC scores obtained in the second PCA revealed that, in Component 1, FLX and GAL(1–15) + FLX-treated groups showed PC scores significantly lower than the control group ($F_{2,15} = 42{,}97$, $P <$ 0.001) (Fig. 4H), while in Component 2, GAL(1–15) + FLX-treated animals showed PC scores significantly lower compared to the rest of the groups ($F_{2,15} = 5034$, $P = 0.0212$) (Fig. 4I).

The findings from the PCA analysis indicate that only the decrease in Kd within the PFC is aligned with the effects induced by the GAL(1–15)-FLX combination in the FST, which involve a reduction in immobility and an increase in swimming time. Additionally, the administration of FLX led to alterations in Kd within the DR, DG ventral hippocampus and modifications in the Bmax parameter across all the examined regions.

3.3. Hormonal effects

3.3.1. Corticosterone levels

The corticosterone levels were examined in the WKY animals one hour after the TST was conducted. Only the coadministration of GAL (1–15) and FLX induced a significant statistical reduction of the corticosterone levels ($F_{2,16} = 4.141$, $P = 0.0355$; Fisher's LSD post hoc: $P <$ 0.001), approximately by 30% in comparison to both the control and FLX-treated WKY group.

3.3.2. DEX/CRH test

After DEX pretreatment, no difference between groups was observed in the corticosterone levels at 18:00 h and 19:00 h. (Fig. 5B).

The administration of CRH stimulation increased plasma corticosterone levels across all groups (Fig. 5B). Repeated measures of one-way ANOVA (with factors being treatment and time) showed a statistically significant elevation in corticosterone levels induced by CRH administration observed across all groups compared to baseline levels. The peak surge in plasma corticosterone after the CRH stimulation 19:30 h occurred promptly at 19:40 h in all groups. Comparable or slightly reduced corticosterone levels were identified at 20:00 h and 20:20 h (Fig. 5B).

Statistical analysis revealed no significant effects induced by FLX or the GAL(1–15) + FLX in this test at any point on the curve analyzed (Fig. 5B).

3.3.3. PCR studies

We described the impact on mRNA expression of proteins associated with the mechanism of the interaction of GAL(1–15) with FLX in the hypothalamus, ventral and dorsal hippocampus and the PFC.

Table 2 compiles the data on mRNA expression levels from WKY rats treated with FLX or GAL(1–15) + FLX. The analysis includes not only a group of WKY saline rats but also a group of Wistar animals across the brain regions of interest.

For the first time, we described the expression levels of GALR1 and GALR2 in WKY strain animals, contrasting them with the outbred Wistar strain rats. Our investigation revealed alterations in expression within two specific regions: the hypothalamus and the ventral hippocampus. Notably, GALR1 exhibited an elevated mRNA expression in the hypothalamus of WKY rats when compared to Wistar animals ($F_{3,17} = 3.87$, P = 0.028). Conversely, in the ventral hippocampus, there was a significant reduction in GALR2 expression in WKY animals relative to the Wistar strain ($F_{3,14} = 8.763$, $P = 0.0016$).

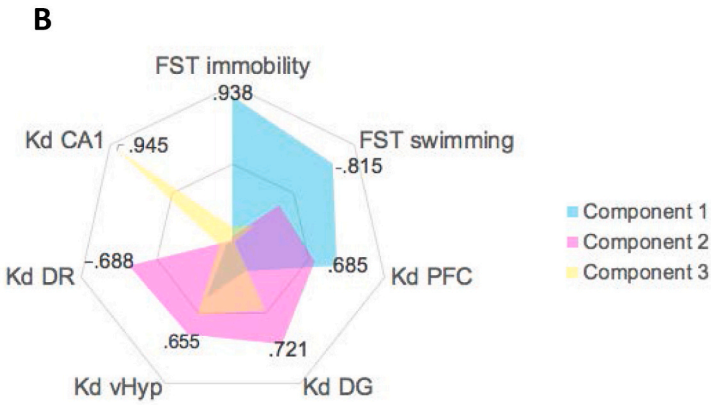
It is interesting to highlight that heightened GALR1 levels in the hypothalamus of WKY returned to baseline values after the coadministration of GAL(1–15) + FLX (Fisher's LSD post hoc: $P < 0.05$), whereas FLX alone did not produce any effect. In the ventral hippocampus, the coadministration of GAL(1–15) + FLX reduced GALR2 levels compared with FLX alone (Fisher's LSD post hoc: $P < 0.05$). In the other brain areas analyzed, no differences were observed in the mRNA levels of GALR1 or GALR2 induced by any treatments.

The analysis also included an examination of the mRNA levels of glucocorticoid (GR) and mineralocorticoid receptors (MR). Notably, we observed a statistically significant rise in GR levels within the hypothalamus of both the WKY control and FLX-treated groups when compared to the Wistar strain ($F_{3,16} = 4.781$, $P = 0.0145$). This increase is reduced with GAL(1–15) + FLX coadministration in the WKY animals.

We also assessed the mRNA expression levels of BDNF and TrkB. In the dorsal hippocampus, there was a notable and statistically significant

A

	Component 1	Component 2	Component 3
FST immobility	**0,938**	0,037	-0,101
FST swimming	**-0,815**	0,38	-0,175
Kd PFC	**0,685**	0,541	0,012
Kd DG	-0,203	**-0,721**	0,483
Kd vHyp	0,403	**-0,655**	0,516
Kd DR	0,043	**0,688**	0,081
Kd CA1	0,003	0,015	**0,945**
Eigenvalue	2,495	1,995	1,031
Variance explained	35,6%	28,5%	14,7%



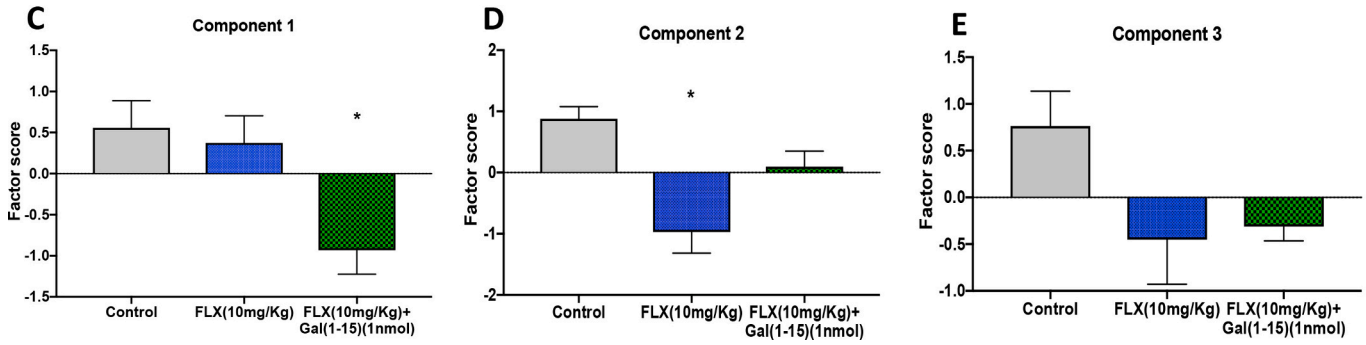


Fig. 3. General dimensions extracted from the FST parameters and the Kd of autoradiographic experiment. (A, B) The PCA revealed three independent components indicative of the FST performance and the Kd values in PFC (C1, variance explained ~35.6%), the Kd measures in the dorsal raphe (DR), the ventral hippocampus (vHip) and the dentate gyrus (DG) of the dorsal hippocampus (C2, variance explained ~28.5%) and the Kd measures in CA1 of the dorsal hippocampus (C3, variance explaines ~14.7%). (C, D and E) Data represent the mean of the factor score of the subjects in each one of the components ± SEM in Control, FLX(10 mg/Kg) and FLX (10 mg/Kg) + GAL(1–15)(1 nmol) groups ($n = 6$). *$p < 0.05$ versus all other groups according to one-way ANOVA followed by Fisher's least significance difference test.

reduction in BDNF levels across all WKY groups compared to Wistar animals, in line with previous findings ($F_{3,18} = 4.654$, $P = 0.014$). This decrease was similarly observed in the ventral hippocampus.

No alterations were detected in the mRNA expression of 5HT1A in any of the analyzed regions.

4. Discussion

Our study reveals a significant discovery: GAL(1–15) can enhance FLX's antidepressant-like effects in WKY animals, offering a potential model for TRD, particularly in despair behaviour tests. Furthermore, our results indicate the possible involvement of GALR2, as evidenced by M871 blocking GAL(1–15)'s actions in the FST. Additionally, our findings highlight the role of 5HT1AR in the interaction between GAL(1–15) and FLX. Specifically, siRNA-induced downregulation of 5-HT1AR blocked the enhancement of antidepressant-like effects by FLX observed with GAL(1–15) in WKY rats.

In both the FST and TST, WKY animals exhibited increased immobility time and decreased climbing compared to Wistar strain animals, consistent with previous reports (Solberg et al., 2001; Steru et al., 1985). These findings reinforce the WKY strain's inclination towards despair behaviour. Moreover, the FLX doses and administration pattern used in this study, as previously documented (López-Rubalcava and Lucki, 2000), did not induce antidepressant-like effects in either test, underscoring the WKY strain's resistance to conventional antidepressants.

In WKY rats, only the GAL(1–15) + FLX combination effectively alleviates behavioural despair, consistent with our previous findings. GAL (1–15) has been demonstrated to enhance the antidepressant effects of FLX in both naïve (Flores-Burgess et al., 2019; Flores-Burgess et al., 2017) and OBX animals (Flores-Burgess et al., 2021). This enhancement was also observed with another SSRI-type antidepressant, Escitalopram, in the OBX model, where GAL(1–15) augmented Escitalopram effects in despair-related behavioural tests (García-Durán et al., 2021). These results confirm the potent effect of combining GAL(1–15) with SSRIs in reversing depressive symptoms, suggesting its potential as augmentation therapy in TRD.

The lack of effect in sucrose preference or consumption between WKY animals and Wistar rats in the SPT aligns with previous studies involving WKY animals (Dommett and Rostron, 2013). However, some authors have reported decreased preference after prior stress exposure (Malkesman et al., 2005). It's important to note the variations in habituation times and sucrose concentrations used across different SPT studies.

Our results suggest the participation of GALR2 in the GAL(1–15)-mediated effects since the GALR2 antagonist M871 blocked in WKY rats the GAL(1–15) enhancement of antidepressant effects of FLX. These results are in consonance with our previous studies showing that GAL (1–15) preferentially binds to GALR1-GALR2 heteroreceptor complexes (Borroto-Escuela et al., 2014; Fuxe et al., 2012; Millón et al., 2017; Millón et al., 2014).

Our study analyzed GALR1 and GALR2 mRNA expression levels in various brain regions, comparing them with outbred Wistar strain rats for the first time. We found region-specific changes in mRNA expression in WKY animals: GALR2 expression decreased in the ventral hippocampus, while GALR1 increased in the hypothalamus. Interestingly, the combination of GAL(1–15) and FLX had different effects in these

F

	Component 1	Component 2
FST immobility	0,114	**0,936**
FST swimming	0,045	**-0,889**
Bmax PFC	**-0,901**	0,105
Bmax GD	**0,624**	0,261
Bmax CA1	**0,883**	0,19
Bmax vHyp	**0,779**	0,122
Bmax DR	**-0,704**	0,074
Eigenvalue	3,11	1,79
Variance explained	44,4%	25,6%

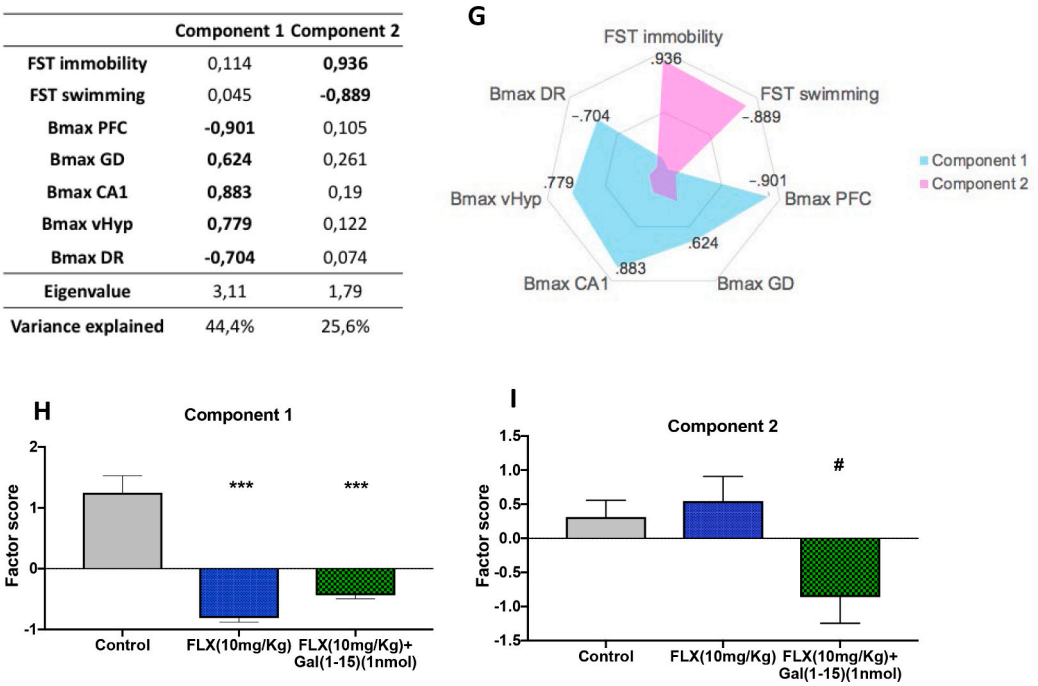


Fig. 4. General dimensions extracted from the FST parameters and the Bmax of autoradiographic experiment. (F, G) The PCA revealed two independent components indicative of Bmax values in PFC, the dorsal raphe (DR), the ventral hippocampus (vHip), and the dentate gyrus (DG) and CA1 of the dorsal hippocampus (C4, variance explained ~44.4%) and the FST performance (C5, variance explained ~25.6%). (H, I) Data represent the mean of the factor score of the subjects in each one of the components ± SEM in Control, FLX(10 mg/Kg) and FLX(10 mg/Kg) + GAL(1–15)(1 nmol) groups ($n = 6$). ***$p < 0.001$ versus control group, #$p < 0.05$ versus FLX(10 mg/Kg) group according to one-way ANOVA followed by Fisher's least significance difference test.

regions. In the hypothalamus, it restored GALR1 levels to baseline, while in the ventral hippocampus, it decreased GALR2 levels compared to FLX alone. These findings highlight the roles of GALR1 and GALR2 in GAL (1–15)-mediated effects.

The critical role of the 5-HT1AR in GAL(1–15)-induced effects was also confirmed at behavioural and neurochemical levels. Thus, the reduction of 5-HT1AR levels, using siRNA 5-HT1AR knockdown in WKY rats, blocks GAL(1–15)'s enhancement of antidepressant-like effects mediated by FLX, consistent with previous findings in both naïve and OBX animals.

In this work, we also investigated the impact of GAL(1–15) + FLX treatment on [3H]-8-OH-DPAT binding characteristics in the mPFC, DR, DG, CA1 of the dorsal hippocampus, and ventral hippocampus of WKY rats, validating the role of 5-HT1AR in GAL(1–15)-mediated effects.

Previous autoradiography experiments in naïve and OBX animals revealed that the combination of GAL(1–15) and FLX modified the Kd and Bmax of 5-HT1AR in the mPFC, a critical region involved in the interplay between emotional processing and cognition or in the dorsal hippocampus CA1 and DG regions. The data obtained in this work also show modifications in the Bmax and Kd in all regions analyzed. However, in the mPFC and dorsal hippocampus DG region, the administration of GAL(1–15) + FLX induce differences with respect to other experimental groups. It is noteworthy to mention that among all the observed changes, the PCA examining the correlation between FST outcomes (immobility, swimming) and Kd/Bmax autoradiographic data revealed that, in our experiments, the prefrontal cortex (PFC) was the only region associated with FST performance. Lower Kd values in the mPFC, indicated by low PC scores, corresponded to reduced immobility and increased swimming during the FST task. We noted a significant decrease in PC scores in the GAL(1–15) + FLX-treated animal group, suggesting that this group exhibited diminished despair behaviour and lower Kd values in the mPFC. However, we must consider when interpreting these results that, although regional differences have been described in the coupling of 5HT1AR with G protein subtypes (Mannoury la Cour et al., 2006), we do not know in the WKY strain if there are modifications that could explain the data obtained in the Kd of the mPCF in this work. Furthermore, we cannot exclude the role of other serotonergic receptors in the interaction between GAL(1–15) + FLX at a behavioural level.

All these data reinforce our previous hypothesis, the existence of a trimeric GALR1-GALR2–5-HT1AR heteroreceptor complex (Borroto-Escuela et al., 2018; Flores-Burgess et al., 2019; Flores-Burgess et al., 2021; Flores-Burgess et al., 2017; Millón et al., 2016) that could be a pivotal point to understanding the effects of GAL(1–15)-SSRI interaction in the WKY. In such complex, altered allosteric receptor-receptor interactions can develop with the ability of the GALR1-GALR2 component to enhance the 5-HT1AR protomer signalling (Flores-Burgess et al., 2019; Flores-Burgess et al., 2017).

Numerous studies have highlighted the heightened susceptibility of WKY strain rats to exacerbated depressive-like behaviour in response to environmental stressors (Millard et al., 2020).This susceptibility may be linked to impaired efficacy of glucocorticoid negative feedback mechanisms, as previously reported (Millard et al., 2020), or prolonged changes in peripheral functions (Redei et al., 1994). Our findings show elevated mRNA expression levels of glucocorticoid receptors (GR) in WKY rats compared to Wistar strain rats. Prior research has also noted variations in GR expression levels across different brain regions in the WKY strain. (Hauger et al., 2002; Shepard and Myers, 2008).

Our findings indicate abnormally elevated corticosterone levels in WKY control rats analyzed one hour after the TST, compared to findings from other studies (Solberg et al., 2001). This consistency aligns with previous reports demonstrating higher corticosterone levels in WKY rats compared to other strains during behavioural tests such as the Open Field Test (Durand et al., 1999) or the FST (De La Garza 2nd and Mahoney 3rd, 2004). Administration of FLX did not significantly affect corticosterone levels in WKY rats under ordinary or stress conditions (Durand et al., 1999). Interestingly, the GAL(1–15) + FLX combination uniquely reduced corticosterone levels, consistent with our previous

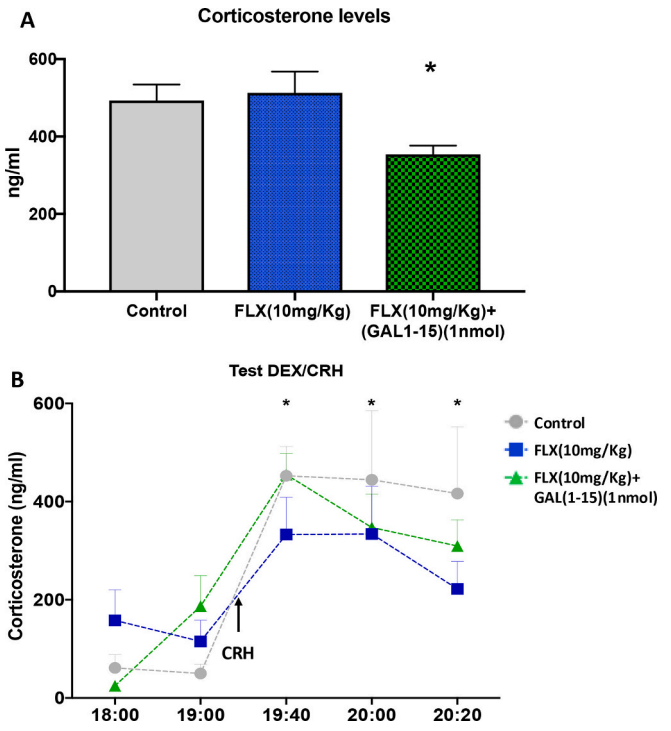


Fig. 5. A) Plasma levels of corticosterone (ng/ml) after the administration of FLX(10 mg/Kg) alone or in combination with GAL(1–15)(1 nmol) in WKY rats. FLX was administered sc 23, 5 and 1 h before performing the TST, and aCSF or GAL(1–15) were injected icv 15 min before. Blood collecting was performed 1 h after the test. Vertical bars represent the mean ± SEM (n = 7–6 rats per group). *$p < 0.05$ versus the rest of the groups according to one-way ANOVA followed by Fisher's least significance difference test. B) Profile of corticosterone levels in plasma (ng/ml) after the administration of FLX(10 mg/Kg) alone or in combination with GAL(1–15)(1 nmol) in WKY rats during DEX/CRH test. FLX was administered sc 23, 5 and 1 h before 19:30 h. aCSF or GAL(1–15) were injected icv 15 min before 19:30 h. All animals was administered iv with DEX (30 μg/Kg) at 12:00 h and CRH (50 ng/kg) at 19:30 h. Blood collecting was performed in the different time points indicated in the graph. A repeated measures one-way ANOVA (treatment x time) was performed. The results showed a statistically significant increase of corticosterone in all groups induced by the administration of CRH in relation with basal levels *$p < 0.05$, but no significance for interaction or treatment effects was obtained.

results in the OBX depression animal model (article OBX). This suggests potential underlying mechanisms involving the regulation of the HPA axis.

To explore potential mechanisms of action involving regulatory elements of the HPA axis in the effects of the GAL(1–15)-FLX combination, we conducted a DEX/CRH test in WKY animals treated with FLX or GAL (1–15) + FLX. The DEX/CRH test combines the dexamethasone suppression test with the CRH challenge and has been extensively utilized to quantify HPA axis dysregulation, showing diagnostic utility in hospitalized depressed patients (Steimer et al., 2007). Adapted for rodents in various depression models (Keck et al., 2002), we employed this test to assess the potential effects of FLX or GAL(1–15) + FLX treatment in WKY rats.

CRH stimulation increased plasma corticosterone levels in all groups, but neither FLX nor GAL(1–15) + FLX showed significant effects at any analyzed point on the curve. Although our experimental design focused on analyzing the possible effects of the treatment on the action of CRH, further studies should analyze whether the treatment can modify the DEX action.

All these novel data deepen the innovative potential that the interaction between the galaninergic and serotonergic systems represents to find new strategies and drugs against resistant depression.

Table 2
Effect of the administration of FLX(10 mg/Kg) alone or in combination with GAL (1–15)(1 nmol) on the mRNA relative expression of Glucocorticoid receptor (GR), Mineralocorticoid receptor (MR), 5ht1a, GalR1, GalR2, BDNF, TrkB, p11 and Homer1a gene expression in the hypothalamus (HPT), ventral (vHyp) and dorsal (dHyp) hippocampus and the prefrontal cortex (PFC) of WKY rats and compared with Wistar strain no-treatment rats. FLX was administered sc 23, 5 and 1 h before performing the TST, and aCSF or GAL(1–15) were injected icv 15 min before. Brains were removed and adequately dissected for PCR experiments 1 h after the test. Data are represented as the mean ± SEM (n = 6–4 rats per group) of the relative expression of genes and are expressed as arbitrary units. *$p < 0.05$ versus Wistar and FLX(10 mg/Kg) + GAL(1–15)(1 nmol). # $p < 0.05$ versus Wistar group, & $p < 0.05$ versus Wistar and FLX(10 mg/Kg) group according to one-way ANOVA followed by Fisher's least significance difference test.

		Wistar	Control	FLX(10 mg/Kg)	FLX(10 mg/Kg) + GAL(1–15)(1 nmol)
HPT	GR	0,57 ± 0,06	0,8 ± 0,063 #	0,79 ± 0,06 #	0,7 ± 0,03
	MR	0,84 ± 0,16	1,09 ± 0,14	0,95 ± 0,12	1,07 ± 0,12
	GalR1	0,69 ± 0,06	0,99 ± 0,12 *	0,97 ± 0,07 *	0,64 ± 0,08
	GalR2	1,01 ± 0,25	0,79 ± 0,07	0,86 ± 0,08	0,82 ± 0,06
	5ht1a	1,01 ± 0,25	0,79 ± 0,07	0,86 ± 0,08	0,82 ± 0,06
vHyp	GR	1,24 ± 0,25	0,81 ± 0,19	0,85 ± 0,2	1,02 ± 0,37
	MR	0,83 ± 0,08	0,69 ± 0,14	1,03 ± 0,12	0,81 ± 0,08
	GalR1	0,47 ± 0,21	1,18 ± 0,24	0,65 ± 0,17	0,52 ± 0,28
	GalR2	3,53 ± 0,52	1,57 ± 0,23 #	1,93 ± 0,44 #	0,53 ± 0,32&
	5ht1a	1,24 ± 0,25	0,81 ± 0,19	0,85 ± 0,2	1,02 ± 0,37
	BDNF	2,32 ± 0,5	1,22 ± 0,3	1,34 ± 0,3	0,99 ± 0,16
	P11	0,59 ± 0,11	0,56 ± 0,13	1,24 ± 0,35	1,1 ± 0,5
dHyp	GR	0,82 ± 0,46	0,94 ± 0,08	0,84 ± 0,35	0,87 ± 0,43
	MR	0,96 ± 0,15	1,22 ± 0,11	1,17 ± 0,2	1,21 ± 0,34
	GalR1	1,47 ± 0,45	1,67 ± 0,9	0,86 ± 0,42	1,8 ± 1,4
	GalR2	1,25 ± 0,27	1,31 ± 0,46	1,46 ± 0,7	1,98 ± 1
	5ht1a	1,01 ± 0,25	0,78 ± 0,11	0,61 ± 0,23	0,58 ± 0,16
	BDNF	1,66 ± 0,35	0,72 ± 0,1 #	0,89 ± 0,31 #	0,39 ± 0,04 #
	TrkB	0,96 ± 0,16	1,88 ± 0,27	1,77 ± 0,44	1,92 ± 0,61
PFC	GR	0,83 ± 0,11	0,75 ± 0,25	0,83 ± 0,3	0,71 ± 0,15
	GalR1	0,82 ± 0,2	0,64 ± 0,22	0,67 ± 0,23	0,83 ± 0,22
	GalR2	1,6 ± 0,28	1,23 ± 0,22	1,4 ± 0,22	1,28 ± 0,31
	5ht1a	0,8 ± 0,08	0,59 ± 0,11	0,56 ± 0,05	0,61 ± 0,1
	BDNF	1,5 ± 0,15	1,73 ± 0,39	1,35 ± 0,33	1,09 ± 0,1
	Homer1a	0,69 ± 0,04	0,73 ± 0,06	0,59 ± 0,06	0,71 ± 0,04

5. Conclusions

In conclusion, our results indicate a potent effect of the combination GAL(1–15) with SSRIs in reversed depressive WKY strain's resistance to conventional antidepressants. The results open up the possibility to use GAL(1–15) in combination with SSRIs as a novel strategy for treatment

of TRD.

Ethical statement

All Experimental procedures and techniques were reviewed and approved by the Institutional Animal Ethics Committee of the University of Málaga, Spain, and were performed in accordance with the EU directive 2010/63/EU. All efforts were made to minimize pain or discomfort as well as the number of animals used during the experiment.

CRediT authorship contribution statement

Juan Pedro Pineda-Gómez: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Carmelo Millón:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Noelia Cantero-García:** Investigation, Formal analysis. **Marta Flores-Gómez:** Investigation, Formal analysis. **David Ladrón de Guevara-Miranda:** Methodology, Investigation, Formal analysis. **Antonio Flores-Burgess:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Zaida Díaz-Cabiale:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

All authors reported no financial interests or potential conflicts of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2024.111191.

References

Aleksandrova, L.R., Wang, Y.T., Phillips, A.G., 2019. Evaluation of the Wistar-Kyoto rat model of depression and the role of synaptic plasticity in depression and antidepressant response. Neurosci. Biobehav. Rev. 105, 1–23. https://doi.org/10.1016/j.neubiorev.2019.07.007.

Artigas, F., Bortolozzi, A., Celada, P., 2018. Can we increase speed and efficacy of antidepressant treatments? Part I: general aspects and monoamine-based strategies. Eur. Neuropsychopharmacol. 28 (4), 445–456. https://doi.org/10.1016/j.euroneuro.2017.10.032.

Bartfai, T., Lu, X., Badie-Mahdavi, H., Barr, A.M., Mazarati, A., Hua, X.Y., Rebek, J., 2004. Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. Proc. Natl. Acad. Sci. U. S. A. 101 (28), 10470–10475. https://doi.org/10.1073/pnas.0403802101.

Borroto-Escuela, D.O., Narvaez, M., Di Palma, M., Calvo, F., Rodriguez, D., Millon, C., Fuxe, K., 2014. Preferential activation by galanin 1-15 fragment of the GalR1 protomer of a GalR1-GalR2 heteroreceptor complex. Biochem. Biophys. Res. Commun. 452 (3), 347–353. https://doi.org/10.1016/j.bbrc.2014.08.061.

Borroto-Escuela, D.O., Narváez, M., Ambrogini, P., Ferraro, L., Brito, I., Romero-Fernandez, W., Fuxe, K., 2018. Receptor¯receptor interactions in multiple 5-HT1A heteroreceptor complexes in raphe-hippocampal 5-HT transmission and their relevance for depression and its treatment. Molecules 23 (6). https://doi.org/10.3390/molecules23061341.

Branchek, T.A., Smith, K.E., Gerald, C., Walker, M.W., 2000. Galanin receptor subtypes. Trends Pharmacol. Sci. 21 (3), 109–117. https://doi.org/10.1016/s0165-6147(00)01446-2.

Chisholm, D., Sweeny, K., Sheehan, P., Rasmussen, B., Smit, F., Cuijpers, P., Saxena, S., 2016. Scaling-up treatment of depression and anxiety: a global return on investment analysis. Lancet Psychiatry 3 (5), 415–424. https://doi.org/10.1016/S2215-0366(16)30024-4.

De La Garza 2nd, R., Mahoney 3rd, J.J., 2004. A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. Brain Res. 1021 (2), 209–218. https://doi.org/10.1016/j.brainres.2004.06.052.

Díaz-Cabiale, Z., Parrado, C., Narváez, M., Millón, C., Puigcerver, A., Fuxe, K., Narváez, J.A., 2010. Neurochemical modulation of central cardiovascular control: the integrative role of galanin. Exp. Suppl. 102, 113–131. https://doi.org/10.1007/978-3-0346-0228-0_9.

Dommett, E.J., Rostron, C.L., 2013. Appetitive and consummative responding for liquid sucrose in the spontaneously hypertensive rat model of attention deficit hyperactivity disorder. Behav. Brain Res. 238, 232–242. https://doi.org/10.1016/j.bbr.2012.10.025.

Durand, M., Berton, O., Aguerre, S., Edno, L., Combourieu, I., Mormède, P., Chaouloff, F., 1999. Effects of repeated fluoxetine on anxiety-related behaviours, central serotonergic systems, and the corticotropic axis axis in SHR and WKY rats. Neuropharmacology 38 (6), 893–907. https://doi.org/10.1016/s0028-3908(99)00009-x.

Fekadu, A., Wooderson, S.C., Markopoulo, K., Donaldson, C., Papadopoulos, A., Cleare, A.J., 2009. What happens to patients with treatment-resistant depression? A systematic review of medium to long term outcome studies. J. Affect. Disord. 116 (1–2), 4–11. https://doi.org/10.1016/j.jad.2008.10.014.

Flores-Burgess, A., Millón, C., Gago, B., Narváez, M., Borroto-Escuela, D.O., Mengod, G., Díaz-Cabiale, Z., 2017. Galanin (1-15) enhancement of the behavioral effects of fluoxetine in the forced swimming test gives a new therapeutic strategy against depression. Neuropharmacology 118, 233–241. https://doi.org/10.1016/j.neuropharm.2017.03.010.

Flores-Burgess, A., Millón, C., Gago, B., García-Durán, L., Cantero-García, N., Coveñas, R., Díaz-Cabiale, Z., 2019. Galanin (1-15)-fluoxetine interaction in the novel object recognition test. Involvement of 5-HT1A receptors in the prefrontal cortex of the rats. Neuropharmacology 155, 104–112. https://doi.org/10.1016/j.neuropharm.2019.05.023.

Flores-Burgess, A., Millón, C., Gago, B., García-Durán, L., Cantero-García, N., Puigcerver, A., Díaz-Cabiale, Z., 2021. Galanin(1-15) enhances the behavioral effects of fluoxetine in the olfatory bulbectomy rat suggesting a new augmentation strategy. Int. J. Neuropsychopharmacol. 25 (4), 307–318. https://doi.org/10.1093/ijnp/pyab089, 2022 Apr 19.

Flores-Burgess, A., Millón, C., Gago, B., García-Durán, L., Cantero-García, N., Puigcerver, A., Díaz-Cabiale, Z., 2022. Galanin (1-15) enhances the behavioral effects of fluoxetine in the olfactory bulbectomy rat, suggesting a new augmentation strategy in depression. Int. J. Neuropsychopharmacol. 25 (4), 307–318. https://doi.org/10.1093/ijnp/pyab089.

Fuxe, K., Marcellino, D., Rivera, A., Diaz-Cabiale, Z., Filip, M., Gago, B., Agnati, L.F., 2008. Receptor-receptor interactions within receptor mosaics. Impact on neuropsychopharmacology. Brain Res. Rev. 58 (2), 415–452. https://doi.org/10.1016/j.brainresrev.2007.11.007.

Fuxe, K., Borroto-Escuela, D.O., Romero-Fernandez, W., Tarakanov, A.O., Calvo, F., Garriga, P., Díaz-Cabiale, Z., 2012. On the existence and function of galanin receptor heteromers in the central nervous system. Front. Endocrinol. (Lausanne) 3, 127. https://doi.org/10.3389/fendo.2012.00127.

García-Durán, L., Flores-Burgess, A., Cantero-García, N., Puigcerver, A., Narváez, J., Fuxe, K., Díaz-Cabiale, Z., 2021. Galanin(1-15) potentiates the antidepressant-like effects induced by escitalopram in a rat model of depression. Int. J. Mol. Sci. 22 (19). https://doi.org/10.3390/ijms221910848.

Hauger, R.L., Shelat, S.G., Redei, E.E., 2002. Decreased corticotropin-releasing factor receptor expression and adrenocorticotropic hormone responsiveness in anterior pituitary cells of Wistar-Kyoto rats. J. Neuroendocrinol. 14 (2), 126–134. https://doi.org/10.1046/j.0007-1331.2001.00752.x.

Keck, M.E., Wigger, A., Welt, T., Müller, M.B., Gesing, A., Reul, J.M., Neumann, I.D., 2002. Vasopressin mediates the response of the combined dexamethasone/CRH test in hyper-anxious rats: implications for pathogenesis of affective disorders. Neuropsychopharmacology 26 (1), 94–105. https://doi.org/10.1016/s0893-133x(01)00351-7.

Kessler, R.C., Bromet, E.J., 2013. The epidemiology of depression across cultures. Annu. Rev. Public Health 34, 119–138. https://doi.org/10.1146/annurev-publhealth-031912-114409.

Kuteeva, E., Hökfelt, T., Wardi, T., Ogren, S.O., 2008. Galanin, galanin receptor subtypes and depression-like behaviour. Cell. Mol. Life Sci. 65 (12), 1854–1863. https://doi.org/10.1007/s00018-008-8160-9.

Lang, R., Gundlach, A.L., Kofler, B., 2007. The galanin peptide family: receptor pharmacology, pleiotropic biological actions, and implications in health and disease. Pharmacol. Ther. 115 (2), 177–207. https://doi.org/10.1016/j.pharmthera.2007.05.009.

López-Rubalcava, C., Lucki, I., 2000. Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. Neuropsychopharmacology 22 (2), 191–199. https://doi.org/10.1016/s0893-133x(99)00100-1.

Lu, X., Lundström, L., Langel, U., Bartfai, T., 2005. Galanin receptor ligands. Neuropeptides 39 (3), 143–146. https://doi.org/10.1016/j.npep.2004.12.012.

Malkesman, O., Braw, Y., Zagoory-Sharon, O., Golan, O., Lavi-Avnon, Y., Schroeder, M., Weller, A., 2005. Reward and anxiety in genetic animal models of childhood depression. Behav. Brain Res. 164 (1), 1–10. https://doi.org/10.1016/j.bbr.2005.04.023.

Mannoury la Cour, C., El Mestikawy, S., Hanoun, N., Hamon, M., Lanfumey, L., 2006. Regional differences in the coupling of 5-hydroxytryptamine-1A receptors to G proteins in the rat brain. Mol. Pharmacol. 70 (3), 1013–1021. https://doi.org/10.1124/mol.106.022756.

McAllister-Williams, R.H., Arango, C., Blier, P., Demyttenaere, K., Falkai, P., Gorwood, P., Rush, A.J., 2020. The identification, assessment and management of difficult-to-treat depression: an international consensus statement. J. Affect. Disord. 267, 264–282. https://doi.org/10.1016/j.jad.2020.02.023.

McAllister-Williams, R.H., Arango, C., Blier, P., Demyttenaere, K., Falkai, P., Gorwood, P., Rush, A.J., 2021. Reconceptualising treatment-resistant depression as difficult-to-treat depression. Lancet Psychiatry 8 (1), 14–15. https://doi.org/10.1016/s2215-0366(20)30516-2.

Millard, S.J., Weston-Green, K., Newell, K.A., 2020. The Wistar-Kyoto rat model of endogenous depression: a tool for exploring treatment resistance with an urgent need to focus on sex differences. Prog. Neuropsychopharmacol. Biol. Psychiatry 101, 109908. https://doi.org/10.1016/j.pnpbp.2020.109908.

Millón, C., Flores-Burgess, A., Narváez, M., Borroto-Escuela, D.O., Santín, L., Parrado, C., Díaz-Cabiale, Z., 2014. A role for galanin N-terminal fragment (1-15) in anxiety- and depression-related behaviors in rats. Int. J. Neuropsychopharmacol. 18 (3). https://doi.org/10.1093/ijnp/pyu064.

Millón, C., Flores-Burgess, A., Narváez, M., Borroto-Escuela, D.O., Santín, L., Gago, B., Díaz-Cabiale, Z., 2016. Galanin (1-15) enhances the antidepressant effects of the 5-HT1A receptor agonist 8-OH-DPAT: involvement of the raphe-hippocampal 5-HT neuron system. Brain Struct. Funct. 221 (9), 4491–4504. https://doi.org/10.1007/s00429-015-1180-y.

Millón, C., Flores-Burgess, A., Narváez, M., Borroto-Escuela, D.O., Gago, B., Santín, L., Díaz-Cabiale, Z., 2017. The neuropeptides Galanin and Galanin(1-15) in depression-like behaviours. Neuropeptides 64, 39–45. https://doi.org/10.1016/j.npep.2017.01.004.

Millón, C., Flores-Burgess, A., Gago, B., Alén, F., Orio, L., García-Durán, L., Díaz-Cabiale, Z., 2019. Role of the galanin N-terminal fragment (1-15) in anhedonia: involvement of the dopaminergic mesolimbic system. J. Psychopharmacol. 33 (6), 737–747. https://doi.org/10.1177/0269881119844188.

Mitsukawa, K., Lu, X., Bartfai, T., 2010. Galanin, galanin receptors, and drug targets. Exp. Suppl. 102, 7–23. https://doi.org/10.1007/978-3-0346-0228-0_2.

Redei, E., Paré, W.P., Aird, F., Kluczynski, J., 1994. Strain differences in hypothalamic-pituitary-adrenal activity and stress ulcer. Am. J. Physiol. 266 (2 Pt 2), R353–R360. https://doi.org/10.1152/ajpregu.1994.266.2.R353.

Shepard, J.D., Myers, D.A., 2008. Strain differences in anxiety-like behavior: association with corticotropin-releasing factor. Behav. Brain Res. 186 (2), 239–245. https://doi.org/10.1016/j.bbr.2007.08.013.

Solberg, L.C., Olson, S.L., Turek, F.W., Redei, E., 2001. Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281 (3), R786–R794. https://doi.org/10.1152/ajpregu.2001.281.3.R786.

Steimer, T., Python, A., Schulz, P.E., Aubry, J.M., 2007. Plasma corticosterone, dexamethasone (DEX) suppression and DEX/CRH tests in a rat model of genetic vulnerability to depression. Psychoneuroendocrinology 32 (5), 575–579. https://doi.org/10.1016/j.psyneuen.2007.03.012.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl) 85 (3), 367–370. https://doi.org/10.1007/bf00428203.

Trivedi, M.H., Daly, E.J., 2008. Treatment strategies to improve and sustain remission in major depressive disorder. Dialogues Clin. Neurosci. 10 (4), 377–384.