THE NEUROPEPTIDES GALANIN AND GALANIN (1-15) IN DEPRESSION-LIKE BEHAVIOURS

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Abstract

Galanin is a 29 amino acid neuropeptide widely distributed in neurons within the central nervous system. Galanin exerts its biological activities through three different G protein-receptors and participates in a number of functions, including mood regulation. Not only Galanin but also Galanin N-terminal fragments like Galanin(1-15) are active at the central level. In this work, we review the latest findings in studies on Galanin and Galanin(1-15) in depression-related behaviours. Our focus is on animal models for depression, and we pay some attention to research data obtained in human studies. Since Serotonin (5-HT), especially through 5-HT1A, and Galanin receptors interact at both pre-and postsynaptic level, the development of drugs targeting potential GAL_1-GAL_2-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons may represent new treatment strategies in depression.

Key words: depression, galanin

Introduction

Mood disorders, including depression and anxiety, are among the most prevalent mental illnesses with high socioeconomic impact (Gelenberg, 2010; Wittchen *et al.*, 2011). Although the underlying mechanisms have not yet been clearly defined in the last decade the importance of the role of neuropeptides, including Galanin (GAL), and/or their receptors in the treatment of stress-related mood disorders is becoming increasingly apparent (Kormos *et al.*, 2013).

GAL is a 29 amino acid neuropeptide (Tatemoto *et al.*, 1983) widely distributed in neurons within the central nervous system (CNS) including raphe nuclei, cerebral cortex and hippocampus (Jacobowitz *et al.*, 2004) among other nuclei involved in mood disorders. Three GAL receptor subtypes ($GAL_{1\text{-}3}$ receptors) have been described (Branchek *et al.*, 2000; Mitsukawa *et al.*, 2008). GAL_1 and GAL_2 receptors, in particular, are found in many regions of the CNS as demonstrated with in situ hybridization, radioligand binding, and immunohistochemical studies (Jacobowitz *et al.*, 2004) and have all high affinity for GAL. GAL_1 and GAL_3 receptors mainly activate inhibitory G proteins Gi/Go, while GAL_2 receptor primarily couples to Gq/G11 to mediate excitatory signaling (Branchek *et al.*, 2000).

The three GAL receptors participate in a number of functions in the CNS including neuroendocrine levels, mood regulation, pain control, cardiovascular functions, addiction and food intake (Lu *et al.*, 2007; Kuteeva *et al.*, 2008; Mitsukawa *et al.*, 2008; Diaz-Cabiale *et al.*, 2010; Juhasz *et al.*, 2014; Lang *et al.*, 2015)

Not only GAL but also the GAL fragments like GAL N-terminal fragment 1-15 [GAL(1-15)] are active in the CNS (Hedlund *et al.*, 1996; Diaz-Cabiale *et al.*, 2005; Diaz-Cabiale *et al.*, 2010; Millon *et al.*, 2015; Millon *et al.*, 2016). Structure-activity studies described that in the brain, N-terminal fragments of GAL are biologically active, whereas C-terminal fragments are inactive (Diaz-Cabiale *et al.*, 1998) and they act as agonists in several physiological functions (Diaz-Cabiale *et al.*, 1998; Diaz-Cabiale *et al.*, 2005), suggesting a receptor-

mediated action. Although the three GAL receptors subtypes show higher affinity for GAL than for GAL(1-15) (Branchek *et al.*, 1998), the presence of specific binding sites for this GAL fragment in the CNS also in areas lacking [^{125}I]- GAL binding sites indicated a powerful role of GAL fragments, especially in the dorsal hippocampus, neocortex and striatum (Hedlund *et al.*, 1992). Only GAL(1-15), but not GAL, can antagonically modulate the serotonin 5-HT1A receptors in the dorsal hippocampus, and this effect was blocked by the GAL receptor antagonist M35 (Hedlund *et al.*, 1994). In the ventral limbic cortex, N-terminal GAL fragments can more stringy and more potently reduce posjunctionals 5-HT1A receptor recognition than GAL, where high-affinity GAL receptors also exist (Diaz-Cabiale *et al.*, 2000; Diaz-Cabiale *et al.*, 2010). The formation of GAL_1/GAL_2 heteroreceptors highly specific for GAL fragments will explain the different effects between GAL and GAL(1-15) (Fuxe *et al.*, 2008; Fuxe *et al.*, 2012; Millon *et al.*, 2015).

In this work, we review the latest findings in studies on GAL and GAL(1-15) in depression-related behaviours. Our focus is on animal models for depression, and we pay some attention to research data obtained in human studies.

GALANIN IN DEPRESSION

A major impediment in depression research is the lack of validated animals´ models. Animals not only lack consciousness of self, self-reflection and consideration of others but also hallmarks of the disorder such as depressed mood, low self esteem or suicidal intent are hardly accessible in non-human (Deussing, 2006). However, depression, as other mental disorders, constitutes of intermediate or so-called endophenotypes that can be reproduced independently and evaluated in animals, including physiological, endocrinological and neuroanatomical alterations as well as behavioural traits. Numerous behavioural paradigms have been established to elucidate face and construct validity of depression models, including antidepressant-screening test (Deussing, 2006).

The forced swimming test (FST), the most used paradigm to assess depression- and antidepressant- like behaviour, also known as the 'behavioural despair' test, was developed by Porsolt et al. (Porsolt *et al.*, 1977) as a rodent model for predicting the clinical efficacy of antidepressant drugs (Bogdanova *et al.*, 2013). The FST takes advantage of the observation that rodents, following initial escape-oriented movements, rapidly adopt a characteristic immobile posture in an inescapable cylinder filled with water. In this paradigm, immobility is interpreted as a passive stress-coping strategy or depression-like behaviour (behavioural despair) (Deussing, 2006).

Fuxe´s Laboratory, around nineties, reported the first evidence of the involvement of GAL in depression. In animal models, GAL administered into the lateral ventricle (i.c.v) reduced serotonin (5-HT) metabolism in ventral limbic cortex, hippocampal formation, and fronto-parietal cortex probably via direct inhibitory actions on dorsal raphe (DR) 5-HT nerve cells reducing their firing rates (Fuxe *et al.*, 1988b). These results suggested based on the 5-HT hypothesis of depression that GAL, may contribute to depression by reducing firing in the ascending 5-HT neurons (Fuxe *et al.*, 1988b).

GAL in the rat ventral tegmental area (VTA) induced in a dose-dependent manner an increased in immobility time in the FST (Weiss *et al.*, 1998), an effect that was blocked by the coinfusion of the GAL receptor antagonist M35. I.c.v. GAL also resulted in an increase of immobility time in FST confirming that GAL may induce depression-like phenotype (Kuteeva *et al.*, 2007). In the rat, intraperitoneal injection of galnon or galmic, GAL receptor agonists, reduced immobility time in the rat FST, indicating an antidepressant-like effect of systemically GAL receptor agonists (Bartfai *et al.*, 2004; Lu *et al.*, 2005) (Table 1).

The role of GAL in depression-like behaviour has been analysed in genetically modified mice. Mice over-expressing GAL under the platelet-derived growth factor-B promoter (GalOE-P) displayed an increased immobility in the FST, suggesting a depression-like behaviour (Kuteeva *et al.*, 2005; Kuteeva *et al.*, 2008). However, Holmes et al. (2005) (Holmes *et al.*, 2005) failed to show such

alterations in GAL over-expressing mice under the dopamine-β-hydroxylase promoter (GalOE-D) and GAL_1 receptor knockout (KO) mice (Table 1). Importantly, GalOE-P but not GalOE-D mice showed an augmentation of hippocampal noradrenaline (NA) and 5-HT release probably indicating that the mechanisms underlying the increase of immobility in GalOE-P can be related to modulation of NA and 5-HT transmission.

In a genetic rat model of depression, the Flinder sensitive line, which displays a high immobility in FST, an up-regulation of the GAL receptor binding sites, is found in the DR, and reduces GAL fiber density in the hippocampus and hypothalamus. The results indicate that enhancement of GAL receptors function in the DR rich in 5-HT neurons could be a mechanism involved in the production of depressive-like activity in this animal model of depression (Bellido *et al.*, 2002).

Galanin Receptors in depression

Several studies suggest that GAL_1/GAL_3 receptors can contribute to the pro-depressive effect of GAL. I.c.v infusion of GAL_1 receptor agonist (M617) elevated the immobility time in the FST, similar to GAL itself. Moreover, the depression-like behavior in rats exposed to chronic mild stress was related to an elevated expression of GAL_1 receptor in the ventral periaqueductal gray (Wang *et al.*, 2016). Furthermore, administration of GAL_3 receptor antagonists to rat and mice produced antidepressant-like effects in both FST and the tail suspension test (TST) (Swanson *et al.*, 2005; Barr *et al.*, 2006) (Table 1). The TST is another prominent test, which relies on similar assumptions and interpretations as the FST (Steru *et al.*, 1985). In the TST, animals are suspended by their tails for a defined period of time, and their immobility is assessed (Deussing, 2006).

Stimulation of the GAL_2 receptor, in contrast, produces antidepressant-like effects. The i.c.v. GAL_2 receptor agonist AR-M1896 decreased the immobility time in the FST, while the GAL_2 receptor antagonist (M871) elevated the

immobility time in the FST (Kuteeva *et al.*, 2008). Recent studies shown that systemically active GAL_2 receptor agonist reduced the immobility time in the FST and in the TST, confirming the potential antidepressant effect of GAL_2 receptor (Saar *et al.*, 2013b; Saar *et al.*, 2013a) (Table 1).

GAL_1 receptor (Holmes *et al.*, 2003), GAL_3 receptor (Brunner *et al.*, 2014) and GAL_2 receptor (Bailey *et al.*, 2007) null mutant displayed an anxiogenic-like phenotype in the elevated plus maze; nevertheless, neither GAL_1 receptor knockout (Holmes *et al.*, 2005) nor GAL_2 receptor knock out (Gottsch *et al.*, 2005) mice differed from their wild-type littermate in the TST. On the contrary, transgenic mouse over-expressing GAL_2 receptor-enhanced, green fluorescent protein constructed under the platelet-derived growth factor-B promotor decreased levels of immobility in the FST (Le Maitre *et al.*, 2011). GAL_3 receptor knock out mice displayed a trend toward decreased immobility time in the TST, indicating a reduction in depression-like behaviour (Brunner *et al.*, 2014) (Table 1).

All these results indicate that stimulation of GAL_2 receptor may be responsible for antidepressant-like effects, whereas GAL_1 and GAL_3 receptors seem to contribute to the prodepressive effects of GAL.

In addition, both chronic treatments with the selective serotonin reuptake inhibitor (SSRI) fluoxetine and electroconvulsive treatment increase GAL mRNA levels in the DR of the rat, accompanied by an increase in GAL_2 receptor, but not GAL_1 receptor, binding sites in this monoaminergic nuclei. Moreover, co-administration of GAL receptors antagonist M40 blocked the behavioural effect of fluoxetine in the FST, suggesting that the antidepressant action of fluoxetine can, at least partially, be related to an increase in GAL-mediated transmission (Lu *et al.*, 2005; Kuteeva *et al.*, 2008). Yamada and co-workers found that after four-week treatment with sertraline, a SSRI, GAL but not GAL_2 receptor was upregulated in the ventral dentate gyrus (Yamada *et al.*, 2013). Moreover, the chronic administration of desipramine, a tricyclic antidepressant, paroxetine, a SSRI, and phenelzine, a monoamine oxidase inhibitor, affected mRNA for GAL and GAL receptors (Rovin *et al.*, 2012). In the VTA, the most marked change in

mRNA produced by these drugs was a decrease in mRNA for GAL_2 receptor (Rovin *et al.*, 2012). However, Venlafaxine, a selective serotonin and noradrenalin reuptake inhibitor, does not alter transcript levels of GAL or its three receptors (Petschner *et al.*, 2016). These results suggest that the effects on GAL and GAL receptors are not uniform among antidepressants and may be related to certain pharmacological properties (Petschner *et al.*, 2016).

Some human data support the involvement of GAL and its receptors in mood disorders. Thus, GAL given intravenously induced antidepressant-like effects, indicated by a suppression of REM sleep in healthy male volunteers (Murck *et al.*, 2004). Recently in a genome wide screening, the single nucleotide polymorphism of GAL gene has been associated with major depression (Wray *et al.*, 2012). Moreover, a work reported that variants in genes for GAL, and its receptors confer an increased risk of depression and anxiety in people who experienced childhood adversity or recent negative life events (Juhasz *et al.*, 2014). Furthermore, significant positive correlation between plasma GAL level and depression severity has been demonstrated suggesting that plasma GAL levels may be an important biomarker for depression (Wang *et al.*, 2014). Recent findings showed that GAL, GAL_1 and GAL_3 receptors mRNA levels were increased in DR nucleus and GAL and GAL_3 receptor in Locus Coeruleus (LC) in brains from depressed persons who had committed suicide (Barde *et al.*, 2016).

All these studies suggest the importance of GAL and its receptors in the pathogenesis of depression.

GALANIN(1-15) IN DEPRESSION

We have analysed the role of GAL(1-15) on the depression-like behaviour using two tests, the FST and the TST.

In the FST, i.c.v. GAL(1-15) 3 nmol significantly increased the immobility time and decreased the time of climbing by 44% and 46% respectively. In addition, this effect was shown with the administration of GAL(1-15) 6 nmol (Millon *et al.*, 2015). The same pattern of response was observed in the TST, GAL(1-15) at the dose of 3 nmol also significantly increased the immobility behaviour recorded during the 6 min of testing (Millon *et al.*, 2015) (Table 1).

These results in both behavioural tests suggest that GAL(1-15) could evoke a strong depression-like behaviour.

When we compared the effects in FST induced by GAL and GAL(1-15), we observed that the increase in the immobility induced by GAL(1-15) was significantly higher than the one induced by GAL (Millon *et al.*, 2015). Moreover, in climbing behaviour, GAL(1-15) also induced a stronger decrease in climbing response compared with GAL (Millon *et al.*, 2015).

In the open field test and in the light-dark box (Prut *et al.*, 2003), we observed that only GAL(1-15) and not GAL modifies the anxiety parameters (Millon *et al.*, 2015) (Table 1). GAL produced anxiolytic-like effects only in animals tested under heightened stress conditions (Barrera *et al.*, 2005; Millon *et al.*, 2015), on contrary, GAL(1-15) induced an anxiogenic-like effect without a stress situation (Millon *et al.*, 2015).

All these results confirm an important role of GAL(1-15) in mood disorders.

Galanin Receptors involved in GAL(1-15)-mediated effect: siRNA GAL_1 receptor or GAL_2 receptor Knockdown rats.

Gene silencing by RNA interference is as a new method of inhibiting the expression of targeted genes and inducing knockdown of associated proteins both in vitro and in vivo (Nakajima *et al.*, 2012). This RNA interference has been applied in experimental investigations in the treatment of illnesses, including neuropsychiatric disorders (Nakajima *et al.*, 2012). Dharmacom Accell siRNA is

one type of naked siRNA modified chemically resulting in robust silencing of selected genes and knockdown of associated proteins (Nakajima *et al.*, 2012).

We used the Accell siRNA system for generating GAL_1 or GAL_2 receptors knockdown (KD) rats to analyze the involvement of GAL receptors in GAL(1-15)-mediated effects.

Rats were i.c.v. injected with 5 µg of Accell Smart pool siRNA GAL_1 receptor or siRNA GAL_2 receptor (Dharmacom), or 5 µl of vehicle (Accell siRNA Delivery Media). We have analysed the reduction produced by siRNAs with a time-course curve performed in siRNA GAL_2 or GAL_1 receptors animals and we examined the mRNA for GAL_2 and GAL_1 receptors in the dorsal hippocampus by real-time quantitative polymesare chain reaction (RT-PCR) in these animals.

In figure 1A, it is shown that a single injection of siRNA GAL_2 receptor induced a strong reduction of mRNA GAL_2 receptor ($p<0.05$) at day 4 and 6 after siRNA treatment (Millon *et al.*, 2015). However, the icv injection of siRNA GAL_2 receptor lacked effect in mRNA GAL_1 receptor expression (Fig. 1B) [unpublished results].

The single injection of siRNA GAL_2 receptor produced the strongest decreased in the receptor expression 8 days after the injection by 35 % in CA1 of the dorsal hippocampus and by 50% in piriform cortex (Millon *et al.*, 2015).

The same pattern of response in mRNA expression was shown after the administration of siRNA GAL_1 receptor. A strong reduction of mRNA GAL_1 receptor at 8 days ($p<0.001$) was produced by siRNA GAL_1 receptor (Fig. 1C) (Millon *et al.*, 2015). Again, the Accell siRNA system is specific, since that injection of siRNA GAL_1 receptor did not modify the mRNA GAL_2 receptor expression (Fig. 1D) [unpublished results].

The Down-regulation of GAL_2 or GAL_1 receptors did not affect any parameters in the FST or in the open field test (OFT) (Table 1). However, this decrease in GAL_2 or GAL_1 receptors was enough to block the depression- and anxiogenic-like effect of GAL(1-15) in both tests (Millon *et al.*, 2015). Thus, GAL(1-15) at

the dose of 3 nmol lacked effect on immobility, climbing and swimming time in the FST in siRNA GAL_2 receptor or in GAL_1 receptor KD rats. In the OFT the same pattern of response was observed, since GAL(1-15) did not affect neither number of entries as the time spent in the central square of open field in GAL_2 receptor or in GAL_1 receptor KD rats (Millon *et al.*, 2015).

The fact that GAL_1 and GAL_2 receptors are needed to obtain GAL(1-15)-effect confirms the hypothesis that GAL(1-15) preferring sites may be formed through the formation of GAL_1/GAL_2 heteroreceptor complexes which lead to conformational changes in their GAL recognition sites converting GAL_1 and/or GAL_2 receptors into GAL recognition fragment preferring binding sites with reduced affinity for GAL (Fuxe *et al.*, 2008; Fuxe *et al.*, 2012). In recent work, Borroto-Escuela et al. analyzed the existence of GAL_1/GAL_2 heteroreceptor complex in co-transfected HEK cells through Bioluminescence Resonance Energy Transfer ($BRET^2$) giving evidence for the existence of GAL_1/GAL_2 heteroreceptor complexes (Borroto-Escuela *et al.*, 2014). A differential role of GAL and GAL(1-15) in signaling cascades Gi/o-Adenylate cyclase (AC)-Protein kinase A (PKA) and Gq-Phospholipase C (PLC)-Protein kinase C (PKC)/Ca^{2+} was observed with cyclic adenosine monophosphate response element (CRE) and nuclear factor of activated T-cells (NFAT) -luciferase reporter gene assays in co-expressing GAL_1 and/or GAL_2 receptors HEK293T cells (Borroto-Escuela *et al.*, 2014).

The formation of GAL_1/GAL_2 heteroreceptor complexes with high affinity for GAL(1-15) can help explain the fact that GAL(1-15) induces a stronger action than GAL at the behavioral level. As described is this review GAL_2 receptor may induce antidepressant actions via Gq/G11 mediated GAL_2 receptor signaling. It seems possible that in the GAL_1/GAL_2 heteroreceptor complex the GAL_2 receptor protomer signals in a different way upon activation by its preferred ligand GAL(1-15) leading to its strong depression-like action. Alternatively, the GAL(1-15) activation of the GAL_1 receptor protomer may via an allosteric receptor-receptor interaction inhibit the Gq/G11 mediated signaling of the GAL_2 receptor protomer and switch it towards Gi/o mediated signaling. In

this way both GAL_1 and GAL_2 receptors protomers become coupled to Gi/o which may lead to the strong depression-like actions observed with GAL(1-15) (Millon *et al.*, 2015). The formation of homodimers and heterodimers among neuropeptide receptors is known (AbdAlla *et al.*, 2005). GAL_1 receptor can form homodimers (Wirz *et al.*, 2005) and heterodimers with 5-HT1A receptors (Borroto-Escuela *et al.*, 2010; Fuxe *et al.*, 2012) and likely with other G-protein coupled receptors.

The existence of GAL_1/GAL_2 heteroreceptors complexes in brain tissue was demonstrated with in situ Proximity Ligation Assay (PLA) to analyze the proximity of GAL_1 and GAL_2 receptors in the dorsal hippocampus and DR nuclei of the KD rats of GAL_2 receptor (Millon *et al.*, 2015). In the siRNA GAL_2 receptor treated animals, PLA- positive red clusters were still observed in the dorsal hippocampus and DR (Millon *et al.*, 2015). However, the number of GAL_1/GAL_2 complex was reduced by 40% in CA1, CA2 and dentate gyrus in dorsal hippocampus and by 60% in the DR nucleus compared with the vehicle group (Millon *et al.*, 2015). In agreement with these results, in the siRNA GAL_2 receptor treated animals the colocalization of GAL_1 and GAL_2 receptors was reduced in the both areas (Millon *et al.*, 2015).

These results strongly indicate that the PLA signals obtained are specific and represent the GAL_1 and GAL_2 heteroreceptor complex. This reduction of the PLA signal was sufficient to block the depression- and anxiogenic-like effects of GAL(1-15), linking them to its actions at the GAL_1/GAL_2 heteroreceptor complex (Millon *et al.*, 2015).

Effects of Galanin on monoamine systems

The physiological/ pathophysiological mechanisms underlying the action(s) of GAL most probably involves modulation of monoaminergic systems, in particular, the LC and DR nuclei. This was further supported by the fact that all three receptors are found in the DR and LC of rats (O'Donnell *et al.*, 1999; Burazin *et al.*, 2000; Mennicken *et al.*, 2002) and that GAL is co-expressed in

almost 40% of the serotonergic neurons in the DR (Xu *et al.*, 1997) and in around 80% of the noradrenergic neurons in the LC (Holets *et al.*, 1988).

I.c.v. GAL produced a reduction in basal NA release in the ventral hippocampus of the awake rat measured by microdialysis, and, significantly attenuated the increase of extracellular hippocampal NA levels evoked by desipramine (Yoshitake *et al.*, 2003). These effects could involve the LC since GAL inhibits LC firing and produces an outward current in rat brain slices through GAL_{1-3} receptors. The application of GAL_{1-3} receptor agonist M961, but not GAL_2 receptor agonist AR-M1896, caused hyperpolarisation of these LC neurons (Seutin *et al.*, 1989; Sevcik *et al.*, 1993; Pieribone *et al.*, 1995; Ma *et al.*, 2001).

Moreover, Grenhoff et al. (Grenhoff *et al.*, 1993) observed that ‘‘burst’’ firing of LC neurons (i.e., rapid firing of LC) released GAL from terminals on axons of LC neurons projecting to the VTA, and that the hyperpolarizing influence of GAL on dopamine (DA) cell bodies in the VTA decreased the activity of these DA neurons. This finding suggested that the hyperactivity of LC neurons observed in depression might bring about such depression-related responses by decreasing the neural activity of dopaminergic cell bodies in the VTA as the result of GAL released from LC-derived terminals in the VTA (Weiss *et al.*, 1998).

In relation with the DR and the 5-HT system, i.c.v. GAL reduced 5-HT metabolism in ventral limbic cortex, hippocampal formation, and fronto-parietal cortex probably via direct inhibitory action on DR nerve cells reducing their firing rates (Fuxe *et al.*, 1988b). This result is in agreement with other works where i.c.v. GAL into vecinity of the DR caused a dose-related and long-lasting inhibition of 5-HT release in the ventral hippocampus measured by microdialysis (Kehr *et al.*, 2002). Moreover, immunohistochemistry experiments showed a strong GAL immunoreactivity staining was observed in the DR after GAL administration, suggesting that the DR was the site of action of GAL on

hippocampal 5-HT release (Kehr *et al.*, 2002). In agreement, i.c.v. GAL attenuates the increase in extracellular levels of 5-HT induced by SSRI citalopram, indicating that this inhibitory action persisted under conditions of serotonergic activation following reuptake inhibition by an SSRI (Yoshitake *et al.*, 2003; Kuteeva *et al.*, 2008). Electrophysiological experiments, where GAL inhibits the firing rate of 5-HT neurons, probably via G protein-coupled inwardly-rectifying potassium channel (GIRK) (Xu *et al.*, 1998), could support these findings.

The 5-HT1A receptor seems to be a key receptor in the GALR-5-HT interaction. In the DR, i.c.v. GAL induced a time-dependent reduction in affinity and an increase in the 5-HT1A autoreceptor density (Razani *et al.*, 2000). At post-synaptic level GAL reduced the affinity of the 5-HT1A receptors in the ventral limbic cortex (Fuxe *et al.*, 1988a; Hedlund *et al.*, 1996). Moreover, in hypothermia, locomotor activity and passive avoidance, i.c.v. GAL blocked post-synaptic 5-HT1A receptor function (Misane *et al.*, 1998; Razani *et al.*, 2001; Kehr *et al.*, 2002). This interaction can in part be due to the existence of GAL_1-5-HT1AR heteroreceptor complexes in discrete brain regions (Borroto-Escuela *et al.*, 2010)

The GAL(1-15) also modified 5-HT system (Millon *et al.*, 2015). Using a rat medullary raphe-derived cell line RN33B, we observed that GAL(1-15) significantly decreased the 5-HT immunoreactivity in the RN33B cells ($p<0.001$) (Fig.2). Interestingly this reduction was stronger than the one induced by GAL ($p<0.001$) (Fig.2) (Millon *et al.*, 2015). This effect of GAL(1-15) on 5-HT immunoreactivity may indicate a possible mechanism contributing to the depression-like actions of GAL(1-15).

In this cell model, we have detected PLA- positive clusters, indicating close proximity of GAL_1 and GAL_2 receptors and the possible formation of GAL_1/$GLAR_2$ heteroreceptor complexes (Millon *et al.*, 2015). Therefore, the formation of GAL_1/GAL_2 heteroreceptor complexes with high affinities for GAL(1-15) can help explain the fact that GAL(1-15) induces a stronger action than GAL in the reduction of 5-HT in the RN33B. Thus, the strong decrease on

5-HT immunoreactivity induced by GAL(1-15) may indicate a mechanism contributing to the depression- like actions of GAL(1-15) (Fig. 2D) (Millon *et al.*, 2015). Recently, we have described that GAL(1–15) enhances the antidepressant effects induced by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) acting on 5-HT1A receptors operating as postjunctional or as autoreceptors, confirming the importance of the interaction GALR-5-HT1A receptors (Millon *et al.*, 2016).

Conclusion

All these data emphasized the role of GAL and its N-Terminal fragment (1-15) in depression. Moreover, GAL(1-15) induces a stronger depressive effect than GAL through GAL_1-GAL_2 heteroreceptor complexes in the raphe-limbic system. Since 5-HT, especially through 5-HT1A, and GAL receptors interact at both pre- and postsynaptic level, the development of drugs targeting potential GAL_1-GAL_2-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons may represent new treatment in depression.

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References

AbdAlla S, Abdel-Baset A, Lother H, el Massiery A, Quitterer U (2005). Mesangial AT1/B2 receptor heterodimers contribute to angiotensin II hyperresponsiveness in experimental hypertension. *J Mol Neurosci* 26**:** 185-192.

Bailey KR, Pavlova MN, Rohde AD, Hohmann JG, Crawley JN (2007). Galanin receptor subtype 2 (GalR2) null mutant mice display an anxiogenic-like

phenotype specific to the elevated plus-maze. *Pharmacol Biochem Behav* 86**:** 8-20.

Barde S, Ruegg J, Prud'homme J, Ekstrom TJ, Palkovits M, Turecki G*, et al.* (2016). Alterations in the neuropeptide galanin system in major depressive disorder involve levels of transcripts, methylation, and peptide. *Proc Natl Acad Sci U S A* 113**:** E8472-E8481.

Barr AM, Kinney JW, Hill MN, Lu X, Biros S, Rebek J, Jr*., et al.* (2006). A novel, systemically active, selective galanin receptor type-3 ligand exhibits antidepressant-like activity in preclinical tests. *Neurosci Lett* 405**:** 111-115.

Barrera G, Echevarria DJ, Poulin JF, Laforest S, Drolet G, Morilak DA (2005). One for all or one for one: does co-transmission unify the concept of a brain galanin "system" or clarify any consistent role in anxiety? *Neuropeptides* 39**:** 289-292.

Bartfai T, Lu X, Badie-Mahdavi H, Barr AM, Mazarati A, Hua XY*, et al.* (2004). Galmic, a nonpeptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. *Proc Natl Acad Sci U S A* 101**:** 10470-10475.

Bellido I, Diaz-Cabiale Z, Jimenez-Vasquez PA, Andbjer B, Mathe AA, Fuxe K (2002). Increased density of galanin binding sites in the dorsal raphe in a genetic rat model of depression. *Neurosci Lett* 317**:** 101-105.

Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF (2013). Factors influencing behavior in the forced swim test. *Physiol Behav* 118**:** 227-239.

Borroto-Escuela DO, Narvaez M, Marcellino D, Parrado C, Narvaez JA, Tarakanov AO*, et al.* (2010). Galanin receptor-1 modulates 5-hydroxtryptamine-1A signaling via heterodimerization. *Biochem Biophys Res Commun* 393**:** 767-772.

Borroto-Escuela DO, Narvaez M, Di Palma M, Calvo F, Rodriguez D, Millon C*, et al.* (2014). Preferential activation by galanin 1-15 fragment of the GalR1 protomer of a GalR1-GalR2 heteroreceptor complex. *Biochem Biophys Res Commun*.

Branchek T, Smith KE, Walker MW (1998). Molecular biology and pharmacology of galanin receptors. *Ann N Y Acad Sci* 863**:** 94-107.

Branchek TA, Smith KE, Gerald C, Walker MW (2000). Galanin receptor subtypes. *Trends Pharmacol Sci* 21**:** 109-117.

Brunner SM, Farzi A, Locker F, Holub BS, Drexel M, Reichmann F*, et al.* (2014). GAL3 receptor KO mice exhibit an anxiety-like phenotype. *Proc Natl Acad Sci U S A* 111**:** 7138-7143.

Burazin TC, Larm JA, Ryan MC, Gundlach AL (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.

Deussing JM (2006). Animal models of depression. *Drug Discovery Today: Disease Models* 3**:** 9.

Diaz-Cabiale Z, Narvaez JA, Finnman UB, Bellido I, Ogren SO, Fuxe K (2000). Galanin-(1-16) modulates 5-HT1A receptors in the ventral limbic cortex of the rat. *Neuroreport* 11**:** 515-519.

Diaz-Cabiale Z, Narvaez JA, Marcos P, Cordon MP, Covenas R, Fuxe K*, et al.* (1998). Galanin and NH2-terminal galanin fragments in central cardiovascular regulation. *Ann N Y Acad Sci* 863**:** 421-424.

Diaz-Cabiale Z, Parrado C, Vela C, Razani H, Covenas R, Fuxe K*, et al.* (2005). Role of galanin and galanin(1-15) on central cardiovascular control. *Neuropeptides* 39**:** 185-190.

Diaz-Cabiale Z, Parrado C, Narvaez M, Millon C, Puigcerver A, Fuxe K*, et al.* (2010). Neurochemical modulation of central cardiovascular control: the integrative role of galanin. *EXS* 102**:** 113-131.

Fuxe K, von Euler G, Agnati LF, Ogren SO (1988a). Galanin selectively modulates 5-hydroxytryptamine 1A receptors in the rat ventral limbic cortex. *Neurosci Lett* 85**:** 163-167.

Fuxe K, Ogren SO, Jansson A, Cintra A, Harfstrand A, Agnati LF (1988b). Intraventricular injections of galanin reduces 5-HT metabolism in the ventral limbic cortex, the hippocampal formation and the fronto-parietal cortex of the male rat. *Acta physiologica Scandinavica* 133**:** 579-581.

Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Tarakanov AO, Calvo F, Garriga P*, et al.* (2012). On the existence and function of galanin receptor heteromers in the central nervous system. *Front Endocrinol (Lausanne)* 3**:** 127.

Fuxe K, Marcellino D, Rivera A, Diaz-Cabiale Z, Filip M, Gago B*, et al.* (2008). Receptor-receptor interactions within receptor mosaics. Impact on neuropsychopharmacology. *Brain Res Rev* 58**:** 415-452.

Gelenberg AJ (2010). The prevalence and impact of depression. *J Clin Psychiatry* 71**:** e06.

Gottsch ML, Zeng H, Hohmann JG, Weinshenker D, Clifton DK, Steiner RA (2005). Phenotypic analysis of mice deficient in the type 2 galanin receptor (GALR2). *Mol Cell Biol* 25**:** 4804-4811.

Grenhoff J, Nisell M, Ferre S, Aston-Jones G, Svensson TH (1993). Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *Journal of neural transmission. General section* 93**:** 11-25.

Hedlund PB, Fuxe K (1996). Galanin and 5-HT1A receptor interactions as an integrative mechanism in 5-HT neurotransmission in the brain. *Ann N Y Acad Sci* 780**:** 193-212.

Hedlund PB, Yanaihara N, Fuxe K (1992). Evidence for specific N-terminal galanin fragment binding sites in the rat brain. *Eur J Pharmacol* 224**:** 203-205.

Hedlund PB, Finnman UB, Yanaihara N, Fuxe K (1994). Galanin-(1-15), but not galanin-(1-29), modulates 5-HT1A receptors in the dorsal hippocampus of the rat brain: possible existence of galanin receptor subtypes. *Brain Res* 634**:** 163-167.

Holets VR, 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.

Holmes A, Li Q, Koenig EA, Gold E, Stephenson D, Yang RJ*, et al.* (2005). Phenotypic assessment of galanin overexpressing and galanin receptor R1 knockout mice in the tail suspension test for depression-related behavior. *Psychopharmacology (Berl)* 178**:** 276-285.

Holmes A, Kinney JW, Wrenn CC, Li Q, Yang RJ, Ma L*, et al.* (2003). Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. *Neuropsychopharmacology* 28**:** 1031-1044.

Jacobowitz DM, Kresse A, Skofitsch G (2004). Galanin in the brain: chemoarchitectonics and brain cartography--a historical review. *Peptides* 25**:** 433-464.

Juhasz G, Hullam G, Eszlari N, Gonda X, Antal P, Anderson IM*, et al.* (2014). Brain galanin system genes interact with life stresses in depression-related phenotypes. *Proc Natl Acad Sci U S A* 111**:** E1666-1673.

Kehr J, Yoshitake T, Wang FH, Razani H, Gimenez-Llort L, Jansson A*, et al.* (2002). Galanin is a potent in vivo modulator of mesencephalic serotonergic neurotransmission. *Neuropsychopharmacology* 27**:** 341-356.

Kormos V, Gaszner B (2013). Role of neuropeptides in anxiety, stress, and depression: from animals to humans. *Neuropeptides* 47**:** 401-419.

Kuteeva E, Hokfelt T, Ogren SO (2005). Behavioural characterisation of young adult transgenic mice overexpressing galanin under the PDGF-B promoter. *Regul Pept* 125**:** 67-78.

Kuteeva E, Wardi T, Hokfelt T, Ogren SO (2007). Galanin enhances and a galanin antagonist attenuates depression-like behaviour in the rat. *Eur Neuropsychopharmacol* 17**:** 64-69.

Kuteeva E, Hokfelt T, Wardi T, Ogren SO (2008). Galanin, galanin receptor subtypes and depression-like behaviour. *Cell Mol Life Sci* 65**:** 1854-1863.

Lang R, Gundlach AL, Holmes FE, Hobson SA, Wynick D, Hokfelt T*, et al.* (2015). Physiology, signaling, and pharmacology of galanin peptides and receptors: three decades of emerging diversity. *Pharmacological reviews* 67**:** 118-175.

Le Maitre TW, Xia S, Le Maitre E, Dun XP, Lu J, Theodorsson E*, et al.* (2011). Galanin receptor 2 overexpressing mice display an antidepressive-like phenotype: possible involvement of the subiculum. *Neuroscience* 190**:** 270-288.

Lu X, Sharkey L, Bartfai T (2007). The brain galanin receptors: targets for novel antidepressant drugs. *CNS & neurological disorders drug targets* 6**:** 183-192.

Lu X, Barr AM, Kinney JW, Sanna P, Conti B, Behrens MM*, et al.* (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.

Ma X, Tong YG, Schmidt R, Brown W, Payza K, Hodzic L*, et al.* (2001). Effects of galanin receptor agonists on locus coeruleus neurons. *Brain Res* 919**:** 169-174.

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.

Millon C, Flores-Burgess A, Narvaez M, Borroto-Escuela DO, Santin L, Parrado C*, et al.* (2015). A role for galanin N-terminal fragment (1-15) in anxiety- and depression-related behaviors in rats. *Int J Neuropsychopharmacol* 18**:** 1-13.

Millon C, Flores-Burgess A, Narvaez M, Borroto-Escuela DO, Santin L, Gago B*, et al.* (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**:** 4491-4504.

Misane I, Razani H, Wang FH, Jansson A, Fuxe K, Ogren SO (1998). Intraventricular galanin modulates a 5-HT1A receptor-mediated behavioural response in the rat. *Eur J Neurosci* 10**:** 1230-1240.

Mitsukawa K, Lu X, Bartfai T (2008). Galanin, galanin receptors and drug targets. *Cell Mol Life Sci* 65**:** 1796-1805.

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.

Nakajima H, Kubo T, Semi Y, Itakura M, Kuwamura M, Izawa T*, et al.* (2012). A rapid, targeted, neuron-selective, in vivo knockdown following a single intracerebroventricular injection of a novel chemically modified siRNA in the adult rat brain. *J Biotechnol* 157**:** 326-333.

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.

Petschner P, Juhasz G, Tamasi V, Adori C, Tothfalusi L, Hokfelt T*, et al.* (2016). Chronic venlafaxine treatment fails to alter the levels of galanin system transcripts in normal rats. *Neuropeptides* 57**:** 65-70.

Pieribone VA, Xu ZQ, Zhang X, Grillner S, Bartfai T, Hokfelt T (1995). Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. *Neuroscience* 64**:** 861-874.

Porsolt RD, Le Pichon M, Jalfre M (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266**:** 730-732.

Prut L, Belzung C (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* 463**:** 3-33.

Razani H, Diaz-Cabiale Z, Fuxe K, Ogren SO (2000). Intraventricular galanin produces a time-dependent modulation of 5-HT1A receptors in the dorsal raphe of the rat. *Neuroreport* 11**:** 3943-3948.

Razani H, Diaz-Cabiale Z, Misane I, Wang FH, Fuxe K, Ogren SO (2001). Prolonged effects of intraventricular galanin on a 5-hydroxytryptamine(1A) receptor mediated function in the rat. *Neurosci Lett* 299**:** 145-149.

Rovin ML, Boss-Williams KA, Alisch RS, Ritchie JC, Weinshenker D, West CH*, et al.* (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, Runesson J, Jarv J, Kurrikoff K, Langel U (2013a). Novel galanin receptor subtype specific ligand in depression like behavior. *Neurochem Res* 38**:** 398-404.

Saar I, Lahe J, Langel K, Runesson J, Webling K, Jarv J*, et al.* (2013b). Novel systemically active galanin receptor 2 ligands in depression-like behavior. *J Neurochem* 127**:** 114-123.

Seutin V, Verbanck P, Massotte L, Dresse A (1989). Galanin decreases the activity of locus coeruleus neurons in vitro. *Eur J Pharmacol* 164**:** 373-376.

Sevcik J, Finta EP, Illes P (1993). Galanin receptors inhibit the spontaneous firing of locus coeruleus neurones and interact with mu-opioid receptors. *Eur J Pharmacol* 230**:** 223-230.

Steru L, Chermat R, Thierry B, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* 85**:** 367-370.

Swanson CJ, Blackburn TP, Zhang X, Zheng K, Xu ZQ, Hokfelt T*, et al.* (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.

Tatemoto K, Rokaeus A, Jornvall H, McDonald TJ, Mutt V (1983). Galanin - a novel biologically active peptide from porcine intestine. *FEBS Lett* 164**:** 124-128.

Wang P, Li H, Barde S, Zhang MD, Sun J, Wang T*, et al.* (2016). Depression-like behavior in rat: Involvement of galanin receptor subtype 1 in the ventral periaqueductal gray. *Proc Natl Acad Sci U S A* 113**:** E4726-4735.

Wang YJ, Yang YT, Li H, Liu PZ, Wang CY, Xu ZQ (2014). Plasma galanin is a biomarker for severity of major depressive disorder. *International journal of psychiatry in medicine* 48**:** 109-119.

Weiss JM, Bonsall RW, Demetrikopoulos MK, Emery MS, West CH (1998). Galanin: a significant role in depression? *Ann N Y Acad Sci* 863**:** 364-382.

Wirz SA, Davis CN, Lu X, Zal T, Bartfai T (2005). Homodimerization and internalization of galanin type 1 receptor in living CHO cells. *Neuropeptides* 39**:** 535-546.

Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jonsson B*, et al.* (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol* 21**:** 655-679.

Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR*, et al.* (2012). Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry* 17**:** 36-48.

Xu ZQ, 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.

Xu ZQ, Zhang X, Pieribone VA, Grillner S, Hokfelt T (1998). Galanin-5-hydroxytryptamine interactions: electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. *Neuroscience* 87**:** 79-94.

Yamada M, Makino Y, Hashimoto T, Sugiyama A, Oka J, Inagaki M*, et al.* (2013). Induction of galanin after chronic sertraline treatment in mouse ventral dentate gyrus. *Brain Res* 1516**:** 76-82.

Yoshitake T, Reenila I, Ogren SO, Hokfelt T, Kehr J (2003). Galanin attenuates basal and antidepressant drug-induced increase of extracellular serotonin and noradrenaline levels in the rat hippocampus. *Neurosci Lett* 339**:** 239-242.

Figure 1. Expression levels of GAL_2 receptor mRNA (A) and GAL_1 receptor mRNA (C) in dorsal hippocampus. (A,B) Expression levels of mRNA GAL_2 or GAL_1 receptors (4 rats per group) measured on different days after administration of the Acell smart pool siRNA for GAL_2 receptor and (C,D) expression levels of mRNA GAL_2 receptor or GAL_1 receptor (4 rats per group) measured on different days after administration of the Acell smart pool siRNA for GAL_1 receptor. The quantitative PCR results were normalized to the expression levels of GAPDH and expressed as arbitrary units. *p<0.05, **p<0.01 vs Vehicle according to student´s t-test. Modified from (Millon *et al.*, 2015).

Figure 2. Close proximity between Galanin receptor 1 (GAL_1 receptor) and Galanin receptor 2 (GAL_2 receptor) and their effects in 5-HT synthesis and storage in RN33B cells. (A) Detection of close proximity between GAL_1-GAL_2 receptors in RN33B cells by *in situ* PLA. Scale bar, 10 μm. (B) Quantification of 5-HT immunoreactivity was analyzed after incubation with GAL and GAL(1-15) ***p<0.001 according to one-way ANOVA followed by Newman Keuls Multiple

Comparison Test. (C) Representative images of 5-HT stained RN33B cells under different conditions are presented. Scale bar, 10 μm. Modified from (Millon *et al.*, 2015). (D) Representative schema illustrating the reduction of 5-HT in the dorsal raphe nuclei induced by the action of GAL(1-15) through the GAL_1/GAL_2 heteroreceptor complexes. Representative projections from DR to cortico-limbic areas are shown in red.

Figure 1

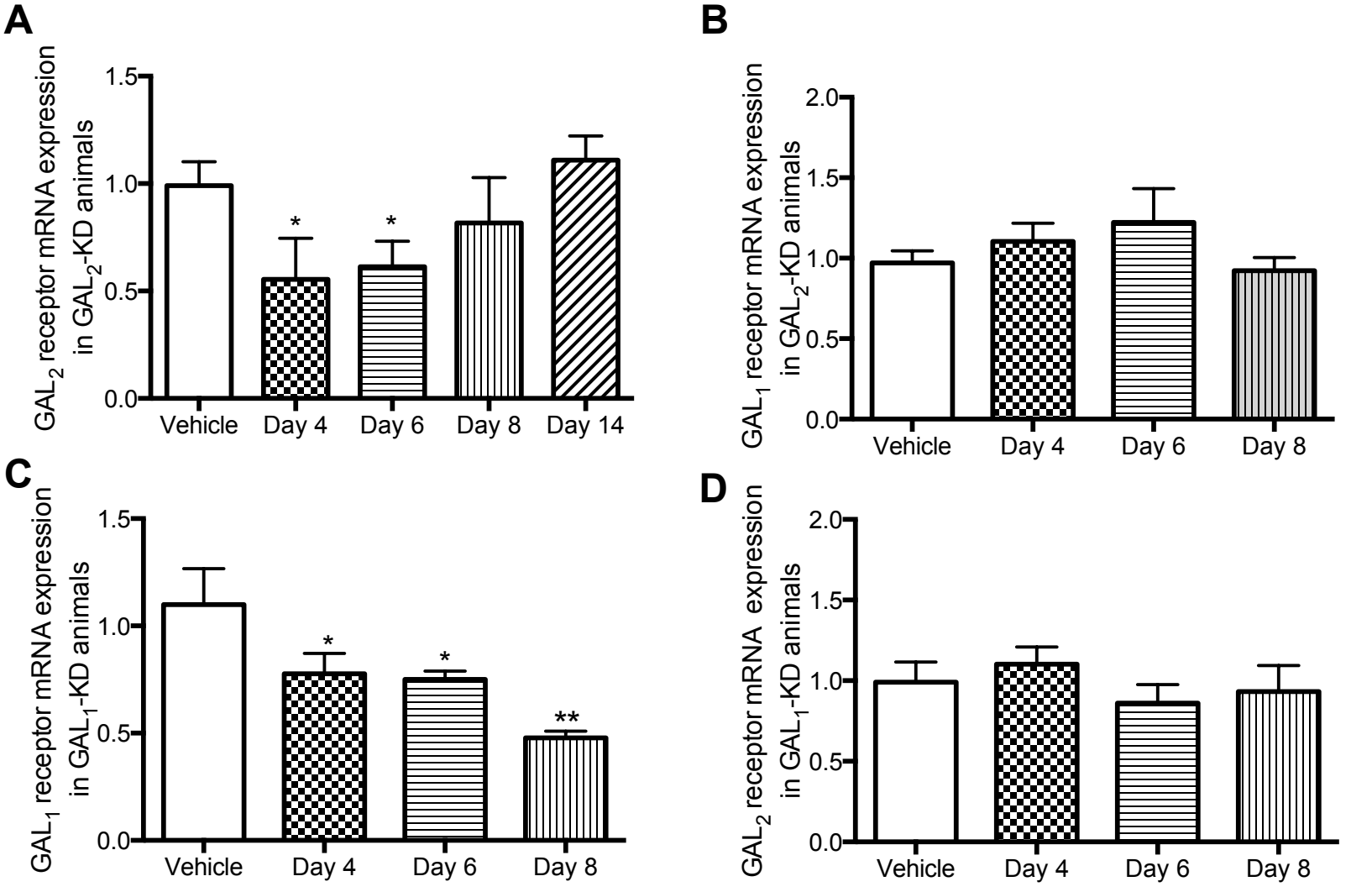
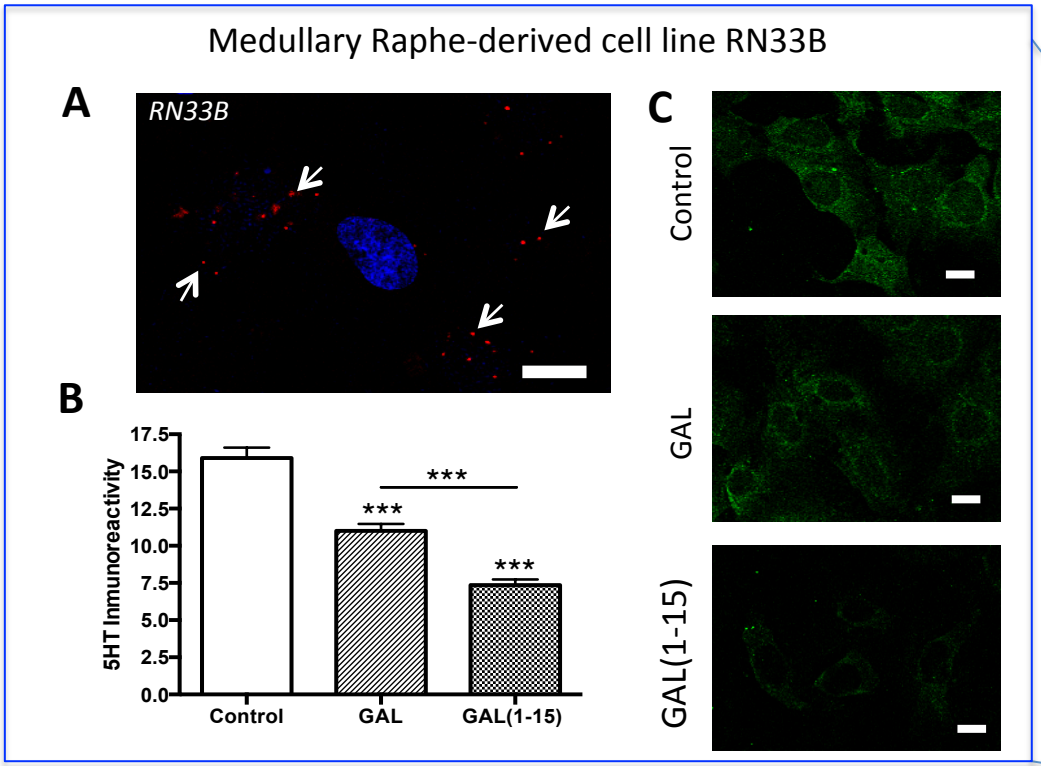
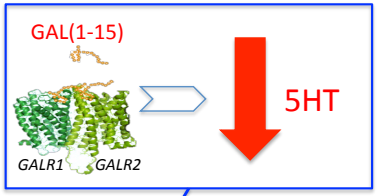
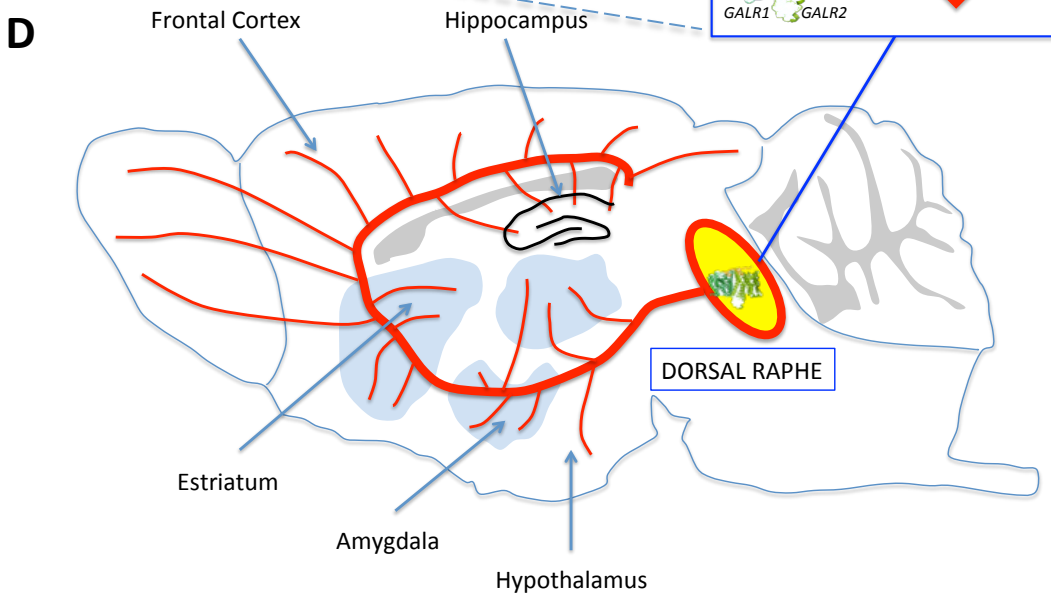

A
GAL2 receptor mRNA expression in GAL2-KD animals
1.5
1.0
0.5
0.0
*
*
Vehicle
Day 4
Day 6
Day 8
Day 14
B
GAL1 receptor mRNA expression in GAL2-KD animals
2.0
1.5
1.0
0.5
0.0
Vehicle
Day 4
Day 6
Day 8
C
GAL1 receptor mRNA expression in GAL1-KD animals
1.5
1.0
0.5
0.0
*
*
**
Vehicle
Day 4
Day 6
Day 8
D
GAL2 receptor mRNA expression in GAL1-KD animals
2.0
1.5
1.0
0.5
0.0
Vehicle
Day 4
Day 6
Day 8

Figure 2


Medullary Raphe-derived cell line RN33B
A
RN33B
B
5HT Immunoreactivity
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
***
***
***
Control
GAL
GAL(1-15)
C
Control
GAL
GAL(1-15)


GAL(1-15)
5HT
GALR1
GALR2


D
Frontal Cortex
Hippocampus
DORSAL RAPHE
Estriatum
Amygdala
Hypothalamus

Ligand	Model	Specie	Route	Effect	Reference
Galanin receptor ligand					
Galanin	FST	Rat	VTA	↑	Weiss et al., 1998
	FST	Rat	i.c.v.	↑	Kuteeva et al., 2007
Galnon	FST	Rat	i.p.	↓	Lu et al., 2005
Galmic	FST	Rat	i.p.	↓	Barfai et al., 2004
M617 (GAL_1 receptor agonist)	FST	Rat	i.c.v.	↑	Kuteeva et a., 2008
SNAP37889 (GAL_3 receptor antagonist)	FST	Rat	i.p.	↓	Swanson et al., 2005
GAL_3 receptor antagonist	TST	Mouse	i.p.	↓	Barr et al., 2006
	FST	Rat	i.p.	↓	Barr et al., 2006
AR-M1896 ($GAL_{2/3}$ receptor agonist)	FST	Rat	i.c.v.	↓	Kuteeva et al., 2008
M871 (GAL_2 receptor antagonist)	FST	Rat	i.c.v.	↓	Kuteeva et al., 2008
M1160 (GAL_2 receptor agonist)	TST	Mouse	i.c.v.	↓	Saar et al., 2013a
J18 (GAL_2 receptor agonist)	FST	Mouse	i.v.	↓	Saar et al., 2013b
GAL(1-15)	FST	Rat	i.c.v.	↑	Millon et al., 2015
	TST	Rat	i.c.v.	↑	Millon et al., 2015
	OFT	Rat	i.c.v.	↑*	Millón et al., 2015
	L/D test	Rat	i.c.v.	↑*	Millon et al., 2015
Genetic Animal Models					
GAL overexpressing (GalOE-P)	FST	Mouse		↑	Kuteeva et al., 2005
GAL overexpressing (GalOE-D)	TST	Mouse		0	Holmes et al., 2005
GAL_1-KO	TST	Mouse		0	Holmes et al., 2005
	EPM	Mouse		↑*	Holmes et al., 2003
GAL_2-KO	EPM	Mouse		↑*	Bailey et al., 2007
	TST	Mouse		0	Gottsch et al., 2005
GAL_2 receptor overexpressing	FST	Mouse		↓	Le Maitre et al., 2011
GAL_3-KO	TST	Mouse		0	Brunner et al., 2014
	FST	Mouse		0	Brunner et al., 2014
	EPM	Mouse		↑*	Brunner et al., 2014
	OFT	Mouse		↑*	Brunner et al., 2014
	L/D test	Mouse		↑*	Brunner et al., 2014
GAL_1-KD	FST	Rat		0	Millon et al., 2015
	OFT	Rat		0	Millon et al., 2015
GAL_2-KD	FST	Rat		0	Millon et al., 2015
	OFT	Rat		0	Millon et al., 2015

Table 1. Effects of Galanin receptor ligands and genetic animal models in rodent test of depression. FST: Forced Swimming Test; TST: Tail Suspension Test; OFT: Open Field Test; EPM: Elevated Plus Maze; L/D Test: Ligth-Dark test; VTA: Ventral Tegmentar Area; i.c.v.: intracerebroventricular; i.p.: intraperitoneal; i.v.: intravenous; KO: Knock Out; KD: Knock Down; ↑: pro-depressive effect; 0: no effect; ↓: antidepressant effec; ↑*: anxiogenic effect.

GALANIN (1-15) AND THE HETERODIMER GALR1/GALR2 IN DEPRESSION AND ANXIETY-LIKE BEHAVIOURS

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Abstract

Galanin is a 29 amino acid neuropeptide widely distributed in neurons within the central nervous system. Galanin exerts its biological activities through three different G protein-receptors and participates in a number of functions, including mood regulation. Not only Galanin but also Galanin N-terminal fragments like Galanin(1-15) are active at the central level. In this work, we review the latest findings in studies on Galanin and Galanin(1-15) in depression-related behaviours. Our focus is on animal models for depression, and we pay some attention to research data obtained in human studies. Since Serotonin (5-HT), especially through 5-HT1A, and Galanin receptors interact at both pre-and postsynaptic level, the development of drugs targeting potential GAL_1-GAL_2-5-HT1A heteroreceptor complexes linked to the raphe-hippocampal 5-HT neurons may represent new treatment strategies in depression.

Key words: depression, galanin

Highlights

- GAL(1-15) produces depression- and anxiety- like effects in behavioural test.
- In these tests, GAL(1-15) induces stronger effects than GAL.
- GALR1/GALR2 heteroreceptor complexes are involved in GAL(1-15)-mediated action.