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Galanin negatively modulates opiate withdrawal via galanin receptor 1

Fiona E. Holmes[1], **Athena Armenaki**[2], **Tiina P. Iismaa**[3], **Emily B. Einstein**[4], **John Shine**[3], **Marina R. Picciotto**[4], **David Wynick**[1], and **Venetia Zachariou**[2]

[1]Schools of Physiology and Pharmacology and Clinical Sciences, University of Bristol, UK.

[2]Department of Pharmacology, University of Crete, Heraklion, Crete, Greece.

[3]Neuroscience Program, Garvan Institute of Medical Research, Sydney, Australia.

[4]Department of Psychiatry, Yale University School of Medicine, New Haven CT, USA.

Abstract

Rationale—The neuropeptide galanin has been shown to modulate opiate dependence and withdrawal. These effects could be mediated via activation of one or more of three distinct G-protein coupled receptors, namely GalR1, GalR2 and GalR3.

Objectives—In this study, we used several transgenic mouse lines to further define the mechanisms underlying the role played by galanin and its receptors in the modulation of morphine dependence. Firstly, transgenic mice expressing β-galactosidase under the control of the galanin promoter were used to assess the regulation of galanin expression in response to chronic morphine administration and withdrawal. Next, the behavioural responses to chronic morphine administration and withdrawal were tested in mice that over-express galanin, lack the GalR1 gene or lack the GalR2 gene.

Methods—Transgenic and matched wild-type mice were given increasing doses of morphine followed by precipitation of withdrawal by naloxone and behavioral responses to withdrawal assessed.

Results—Both morphine administration and withdrawal increases galanin gene transcription in the locus coerulus (LC). Increasing galanin levels in the brain reduced signs of opiate withdrawal. Mice lacking GalR1 undergo more severe opiate withdrawal, whereas mice lacking GalR2 show no significant difference in withdrawal signs, compare to matched wild type controls.

Conclusions—Opiate administration and withdrawal increase galanin expression in the LC. Galanin opposes the actions of morphine which lead to opiate dependence and withdrawal, an effect that is mediated via GalR1.

Keywords

Galanin; galanin receptor 1; mouse; opiate; addiction; withdrawal; locus coeruleus

Authors for correspondence: V. Zachariou, University of Crete, Faculty of Medicine, Department of Pharmacology, Heraklion, Greece, 71005. Tel: 2810-394527; vzachar@med.uoc.gr and D. Wynick, Schools of Physiology and Pharmacology and Clinical Sciences, University of Bristol, UK. Tel: 0117 3311417; d.wynick@bris.ac.uk.

Introduction

The neuropeptide galanin is widely expressed in the hypothalamic-pituitary-adrenal axis and in the central nervous system, including areas of the limbic system and regions of the brain that are important for mediating reward, motivational, dependence and withdrawal responses to drugs of abuse, such as the hypothalamus, hippocampus and locus coeruleus (LC), (Melander et al., 1986, (Koob and Volkow, 2010). Consistent with its distribution in these brain areas, increasing evidence demonstrates that galanin plays an important role in modulating the neurochemical and behavioral correlates of addiction (Picciotto, 2008;Picciotto et al., 2010).

The physiological effects of galanin are mediated by the activation of one or more of three distinct G-protein-coupled receptors, namely GalR1, GalR2 and GalR3 (Burgevin et al., 1995;Kolakowski, Jr. et al., 1998;Wang et al., 1997).

Galanin binding sites are present in areas of the rat brain important for mediating the effects of drugs of abuse (Skofitsch et al., 1986), and GalR1 and GalR2, and to a lesser extent, GalR3 mRNAs, have distinct but overlapping distributions in the limbic system (Mennicken et al., 2002;O'donnell et al., 1999).

Several studies have shown a role for galanin in drug reward-related behaviors. Administration of galanin into the lateral ventricles attenuates the development of conditioned place preference for morphine (Zachariou et al., 1999), and galanin knockout mice are more sensitive to the locomotor stimulant properties of morphine and show increased morphine conditioned place preference compared to matched wild-type (WT) mice (Hawes et al., 2008). Galanin also modulates the behavioral response to psychostimulants such as amphetamine and cocaine (Brabant et al., 2010;Kuteeva et al., 2005;Narasimhaiah et al., 2009). Galanin also appears to modulate opiate dependence and withdrawal. The neuropeptide is expressed in most of the noradrenergic neurons of the LC (Xu et al., 1998), and expression is increased after chronic morphine administration and withdrawal (McClung et al., 2005). *In vitro* studies have shown that galanin decreases the firing of LC neurons by hyperpolarising LC noradrenergic neurons (Mennicken et al., 2002;Rasmussen et al., 1990;Seutin et al., 1989;Sevcik et al., 1993), an effect hypothesized to decrease signs of opiate withdrawal. *In vivo* administration of the small molecule galanin receptor agonist, galnon, alleviates morphine withdrawal symptoms. Furthermore, behavioral signs of withdrawal are exacerbated in galanin knockout mice and attenuated in mice which over-express galanin in NA neurons using the dopamine-β-hydroxylase promoter (Zachariou et al., 2003).

Here, we use several genetically modified mouse lines to further investigate the mechanism underlying the ability of galanin to modulate morphine physical dependence and the expression of opiate withdrawal. In the absence of galanin receptor selective compounds that can be administered systemically, these lines provide a useful tool for understanding the mechanism through which galanin mediates its actions. The current study provides evidence that galanin opposes morphine dependence and withdrawal via a mechanism that involves activation of GalR1 receptors. Thus, GalR1 receptor specific agonists may provide new pharmacological treatments for opiate dependence and withdrawal.

Methods

Animals

All animals were fed standard chow and water ad libitum and housed under identical conditions. Principles of laboratory animal care were followed, and animal care and

procedures were performed within the UK Home Office and University of Crete protocols and guidelines. Adult (~10 week old) male mice (~25g) were used in each experiment with strain, sex and age-matched WTs, derived from the same colony (see below), as controls. Mouse lines were generated as follows:

Galanin-LacZ mice (Gal-LacZ mice)

Details of the transgene construction, generation, strain and breeding history of these mice have been described previously (Bacon et al., 2007). Briefly, Gal-LacZ mice were generated using a ~25kb transgene containing the entire murine galanin genomic locus with ~20kb of the upstream sequence, with a 3.5 kb *LacZ* gene inserted into the 5'-UTR 13 bp downstream of the transcriptional start site. The transgene has been maintained on the CBA X C57BL6F1 hybrid background. Expression of transgene-derived β-galactosidase (LacZ) recapitulates the expression pattern of endogenous galanin.

Galanin overexpressing mice (GalOE mice)

Details of the generation, strain and breeding history have been described previously (Bacon et al., 2007;Elliott-Hunt et al., 2004). In brief, Gal OE mice were generated using a ~25 kb transgene containing the entire murine galanin coding region and 19.9 kb of upstream sequence. Transgenic lines were bred to homozygosity and have been maintained on the CBA X C57BL6F1 hybrid background. The transgenic line denoted OE46 used in this study has high levels of galanin expression in the brain (see (Bacon et al., 2007), for further details).

Galanin Receptor 1 mutant mice (GalR1 mut)

were generated as described previously (Jacoby et al., 2002). Briefly, a targeting vector containing the majority of exon 1 of the murine *GalR1* gene was used to transfect the W9.5 ES cell line, to generate mice that carry an insertional inactivating mutation within the first exon of the GalR1 gene. Mice remain inbred on the 129T2/SvEmsJ strain.

Galanin Receptor 2 mutant mice (GalR2 mut)

Details of the generation, strain and breeding history have been described previously (Hobson et al., 2006). In brief, mice deficient for the *GalR2* gene were generated by and licensed from Lexicon Genetics. The 5.17 kb gene-trap vector VICTR48 (Viral Construct for TRapping) was inserted within the single intron of the murine *GalR2* gene in a 129Sv/EvBrd ES cell-line clone (Zambrowicz et al., 2003). Omnibank clone OST105469 was used to obtain germ-line transmission of the disrupted *GalR2* allele. Heterozygote pairs on the 129/SvEvBrd X C57BL6 background were then bred to homozygosity and maintained on that background.

Transgenic or mutant founders were bred with WTs of the same strain to generate heterozygous mice. Heterozygous mice were then bred to generate homozygous WT and transgenic or mutant mice. Subsequent generations of mice were obtained from homozygous pairings.

Opiate withdrawal

For opiate withdrawal assays, mice were injected with increasing morphine doses (from 20 to 100 mg/kg), every 8 hours for 3 days, and withdrawal was precipitated using naloxone hydrochloride (1 mg/kg, sub-cutaneously (s.c.), Sigma), three hours after the last morphine injection. Withdrawal signs (jumps, wet dog shakes, tremor, ptosis, paw tremor, mastication, backward walking, diarrhea, weight loss) were monitored for 30 min after naloxone

administration (Zachariou et al., 2003). The global withdrawal score was calculated based on the following formula:

(number of backward walking steps × 0.1) + (number of jumps × 0.1) + (ptosis) + (tremor) + (% weight loss) + (diarrhea) + (number of wet dog shakes).

Locomotor activity assay

Mice were habituated to handling for three days prior to locomotor testing. On test days, mice were placed in the testing room for 30 minutes of habituation. Locomotion was measured in 20 cm × 20 cm boxes from Med Associates (ENV-256C Med Associates, Inc, St. Albans, VT, USA). For saline response sessions, mice were injected s.c. immediately before being confined to the chamber for 30 min. For morphine response sessions, mice were injected with morphine (3 or 5mg/kg) s.c. immediately before being confined to the chamber for 30 min to determine early phase locomotor activity. Movement of each mouse was measured by photocell beam breaks and was recorded with Med-PC IV software. One saline and one morphine session were carried out each day for three days; the three locomotor session values for each mouse were averaged and normalized to saline response session values in WT mice.

Immunohistochemistry

For immunohistochemical studies, mice were perfused with 4% paraformaldehyde as described previously (Zachariou et al., 2003). Briefly, 40 μm coronal brain sections were washed in PBS (pH 7.5), incubated in blocking buffer (3% Normal Donkey Serum, 0.3% Triton X-100) for one hour and with rabbit β-gal primary antiserum (1:8000, Chemicon AB1211) overnight. The next day, slices were washed 3 times in PBS, incubated with Cy2 anti-rabbit IgG (1:400 Jackson, PA), washed three times with PBS, mounted, dehydrated and cover-slipped. Images were acquired with a Zeiss microscope. Numbers of LacZ positive nuclei were averaged from the left and right side of LC sections from each animal. All sections were quantified on the same day under the same conditions by an experimenter blind to the treatment groups.

Results

To investigate the effect of chronic morphine treatment and withdrawal on the modulation of galanin expression in the CNS, we used transgenic mice expressing LacZ under the control of a 20.5 kb fragment of the galanin promoter (Bacon et al., 2007). This allowed us to study the effects of morphine on galanin promoter activation. LacZ expression was examined in 3 groups of animals: The first group was treated with saline and the other 2 groups of animals received increasing doses of morphine. Three hours after the last treatment, animals received an injection of naloxone (NLX) or saline. The three groups were a) Saline-NLX, b) Morphine-Saline c) Morphine-NLX. As shown in Fig. 1b, repeated morphine administration results in an increase in LacZ expression in the LC in Gal-LacZ mice. Fig. 1c shows that LacZ expression in the LC is further enhanced following withdrawal. As shown in Fig 1d, there was a 283±75% increase in the number of LacZ positive nuclei following opiate withdrawal, compared to control animals. Morphine produces a small but not significant decrease in the number of LacZ nuclei in the LC. The observed activation of the galanin promoter by morphine and morphine withdrawal is selective, since no change in LacZ expression was observed in the hippocampus (not shown).

We next examined the consequences of galanin over-expression on the behavioral signs of morphine dependence and withdrawal. We used a previously characterized galanin over-expressing transgenic line (OE46) in which high levels of galanin were observed in many brain regions. Galanin over-expressing mice (GalOE) and their matched WT controls were

evaluated in the opiate withdrawal paradigm. As shown in Fig. 2, and consistent with our earlier findings (Bacon et al., 2007;Zachariou et al., 2003), galanin over-expression leads to a milder withdrawal syndrome, as several withdrawal signs, i.e. jumps, wet dog shakes and backward walking are significantly reduced. The global withdrawal score is 26±3 for WT controls and 18.3±2 for GalOE ($p<0.05$, t-test).

The last part of the study sought to determine which galanin receptor subtype mediates the actions of galanin on morphine dependence. As both GalR1 and GalR2, and to a lesser extent GalR3, are expressed in the LC, we used mice lacking a functional GalR1 (GalR1 mut) or GalR2 (GalR2 mut) gene and their respective matched WT controls in the morphine withdrawal paradigm. To date a GalR3 knockout has not been described. Our findings indicate that mice lacking GalR1 exhibit more severe withdrawal behavior compared to their matched WT controls. In particular, GalR1 mut mice show significantly increased jumps, tremor, paw tremor, ptosis and diarrhea (Fig 3; Global withdrawal scores for GalR1 WT = 21.7±2 and for GalR1 mut = 30.3±1, $p<0.05$, t test).

Analysis of GalR2 mutant mice in the same behavioral paradigm indicates that this receptor subtype is not critical for opiate withdrawal, as there is no difference between GalR2 mutants and matched WTs in morphine withdrawal behavioral symptoms. Global withdrawal score for GalR2 WT=18.4±1 and for GalR2 mut=16.6±2.3).

Notably, GalR1 and GalR2 mutant mice show similar locomotor activation to their matched WT controls after morphine administration (Fig. 5), at least during the initial 30 min phase of locomotor activity. However, these results do not preclude a potential influence of GalR1 or GalR2 on later morphine-induced locomotor activity.

GalR1 and GalR2 mutant mice (GalR1 mut, GalR2 mut) and their respective matched wild-type controls (WT) were injected with morphine and their locomotion was measured over thirty minutes. All mice showed comparable beam breaks regardless of genotype. Data are expressed as a percent of average saline beam breaks ± SEM.

Discussion

The neuropeptide galanin and its receptors are widely expressed in the brain and are present in networks known to be important for development and expression of opiate withdrawal (Melander et al., 1986;O'donnell et al., 1999). GalR1, GalR2 and GalR3 couple to $G_{i/o}$, inhibit adenylyl cyclase and decrease cAMP levels, and activate the GIRK family of potassium channels (Habert-Ortoli et al., 1994;Smith et al., 1998). GalR2 also signals via $G_{q/11}$ to activate phospholipase C and protein kinase C (Branchek et al., 2000).

Galanin can modulate neuronal activity in brain regions related to drug dependence and withdrawal. The neuropeptide is expressed in most of the noradrenergic neurons of the LC (Xu et al., 1998), and is increased after chronic morphine administration and withdrawal (McClung et al., 2005). Studies *in vitro* have shown that galanin decreases the firing of LC neurons by hyperpolarizing LC noradrenergic neurons (Rasmussen et al., 1990;Seutin et al., 1989;Sevcik et al., 1993), an effect hypothesized to decrease signs of opiate withdrawal. This has been proposed to be due to a GalR1-mediated increase in membrane permeability to potassium ions (Pieribone et al., 1995).

The current dataset demonstrates that galanin expression is regulated in the LC under conditions leading to morphine dependence and withdrawal, and shows that GalR1 is critical for mediating the effects of galanin to reduce opiate withdrawal signs. Therefore, these studies identify GalR1 as a novel target for the treatment of opiate dependence and withdrawal. This study complements earlier work on the ability of the galanin system to

oppose the behavioral correlates of morphine dependence and withdrawal (Zachariou et al., 2003).

Opiate dependence and withdrawal produce adaptive changes in the cAMP pathway, and one of the most robust changes is increased adenylyl cyclase levels, and thus cAMP production, in the noradrenergic LC neurons (Kogan et al., 1992). *In vitro* studies show that galanin inhibits cAMP formation following chronic morphine administration and withdrawal (Hawes et al., 2006). The first evidence for a potential role of the GalR1 subtype in the actions of galanin on opiate withdrawal came from *in situ* hybridization studies showing up-regulation of this receptor in LC neurons following chronic morphine administration and withdrawal (Zachariou et al., 2000). Furthermore, GalR1 expression in the LC is also modulated by galanin signaling. Both the opiate administration/withdrawal-induced up-regulation of GalR1 and the modulation of GalR1 levels by galanin occur through a cAMP-dependent mechanism involving phosphorylation of the transcription factor cAMP regulatory element-binding protein (CREB) that regulates the GalR1 promoter (Zachariou et al., 2001;Hawes et al., 2005). Here, we show that transcription of the galanin peptide is also increased in LC neurons following opiate dependence and withdrawal. Consistent with the opponent process theory (Koob and Le, 2008;Koob and Volkow, 2010) this up-regulation, along with an increase in GalR1, may be part of a compensatory mechanism to oppose both the increased firing, and cAMP production, of the LC neurons following chronic morphine administration and withdrawal (Hawes et al., 2005;Zachariou et al., 2000). Consistent with previous findings that application of the galanin agonist galnon alleviates several signs of opiate withdrawal (Zachariou et al., 2003), we show that galanin over-expression in the LC leads to a milder withdrawal syndrome, most likely mediated via GalR1. Interestingly, in naïve animals, galanin appears to have a different effect on stress mediated responses, as galanin over-expression leads to augmented stress evoked noradrenaline release (Kehr et al., 2001). We speculate that galanin has distinct roles in stress responses in naïve versus morphine dependent mice. Also, as mentioned above, galanin-mu opioid receptor interactions in the LC are critical for the modulation of LC firing (Sevick et al., 1993). It is possible that GalR-mu opioid receptor dimers can be formed and might have distinct signaling properties that would be abolished in GalR1 knockout mice, resulting in increased LC firing in response to noradrenaline, and a more severe withdrawal phenotype, but this remains to be explored at the molecular level.

Our behavioral data using genetically modified mice, lacking GalR1 or GalR2, show that GalR1 is selectively involved in regulating in opiate withdrawal behaviors, as mice lacking this receptor subtype show significantly more exaggerated symptoms of morphine withdrawal than their WT controls, whereas the GalR2 knockout mice show no overall significant withdrawal phenotype. However, although not statistically significant, there is some reduction in jumps, wet dog shakes and ptosis, which might be attributed to compensatory changes in GalR1 receptor expression/function in GalR2 mutants. These behavioral studies were conducted on several genetically modified mouse lines and their respective WT controls. Transgenic or mutant founder animals were paired with WTs of the same strain to generated heterozygotes, from which were bred homozygous transgenic or mutant mice and their respective WTs. Therefore, although these studies did not use littermates derived from continuous heterozygous breeding (which can be a limitation when interpreting behavioral data, but avoids the generation of unwanted mice) the WTs used were strain, age and sex-matched and housed in an identical environment. Our findings are in agreement with previous knowledge and are coherent between the various genetically modified mice used in this study. These studies help clarify the function of galanin receptor subtypes in morphine dependence and withdrawal and provide important information for new pharmacological strategies for the treatment of morphine dependence and addiction.

Several lines of evidence including *in situ* hybridization, gene array analysis, pharmacological and behavioral studies indicate an important role for the galanin peptide and GalR1 receptor subtype in opiate reward, dependence and withdrawal (Picciotto, 2008). In spite of the importance of alleviating withdrawal to help addicts achieve abstinence, very few drugs to date are available to treat opiate withdrawal symptoms. Compounds targeting α2 adrenergic receptors, such as clonidine, show limited success and have significant side effects. Thus, GalR1 agonists may provide a novel approach to alleviate opiate withdrawal.

In summary, the current set of studies provides novel information about the mechanisms underlying the actions of galanin in opiate dependence and withdrawal and point to GalR1 as a potential new pharmacological target.

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Abbreviations

CREB	cAMP regulatory element-binding protein
lacZ	β-galactosidase
GalR1	galanin receptor 1
GalR2	galanin receptor 2
GalR3	galanin receptor 3
LC	locus coeruleus
WT	wild-type

References

Bacon A, Kerr NC, Holmes FE, Gaston K, Wynick D. Characterization of an enhancer region of the galanin gene that directs expression to the dorsal root ganglion and confers responsiveness to axotomy. J Neurosci. 2007; 27:6573–6580. [PubMed: 17567818]

Brabant C, Kuschpel AS, Picciotto MR. Locomotion and self-administration induced by cocaine in 129/OlaHsd mice lacking galanin. Behav Neurosci. 2010; 124:828–838. [PubMed: 21038934]

Branchek TA, Smith KE, Gerald C, Walker MW. Galanin receptor subtypes. Trends Pharmacol Sci. 2000; 21:109–117. [PubMed: 10689365]

Burgevin MC, Loquet I, Quarteronet D, Habert-Ortoli E. Cloning, pharmacological characterization, and anatomical distribution of a rat cDNA encoding for a galanin receptor. J Mol Neurosci. 1995; 6:33–41. [PubMed: 8562318]

Elliott-Hunt CR, Marsh B, Bacon A, Pope R, Vanderplank P, Wynick D. Galanin acts as a neuroprotective factor to the hippocampus. Proc Natl Acad Sci U S A. 2004; 101:5105–5110. [PubMed: 15041741]

Habert-Ortoli E, Amiranoff B, Loquet I, Laburthe M, Mayaux JF. Molecular cloning of a functional human galanin receptor. Proc Natl Acad Sci U S A. 1994; 91:9780–9783. [PubMed: 7524088]

Hawes JJ, Brunzell DH, Narasimhaiah R, Langel U, Wynick D, Picciotto MR. Galanin protects against behavioral and neurochemical correlates of opiate reward. Neuropsychopharmacology. 2008; 33:1864–1873. [PubMed: 17957220]

Hawes JJ, Brunzell DH, Wynick D, Zachariou V, Picciotto MR. GalR1, but not GalR2 or GalR3, levels are regulated by galanin signaling in the locus coeruleus through a cyclic AMP-dependent mechanism. J Neurochem. 2005; 93:1168–1176. [PubMed: 15934937]

Hawes JJ, Narasimhaiah R, Picciotto MR. Galanin attenuates cyclic AMP regulatory element-binding protein (CREB) phosphorylation induced by chronic morphine and naloxone challenge in Cath.a cells and primary striatal cultures. J Neurochem. 2006; 96:1160–1168. [PubMed: 16417577]

Hobson SA, Holmes FE, Kerr NC, Pope RJ, Wynick D. Mice deficient for galanin receptor 2 have decreased neurite outgrowth from adult sensory neurons and impaired pain-like behaviour. J Neurochem. 2006; 99:1000–1010. [PubMed: 17076662]

Jacoby AS, Hort YJ, Constantinescu G, Shine J, Iismaa TP. Critical role for GALR1 galanin receptor in galanin regulation of neuroendocrine function and seizure activity. Brain Res Mol Brain Res. 2002; 107:195–200. [PubMed: 12487125]

Kehr J, Yoshitake T, Wang FH, Wynick D, Holmberg K, Lendahl U, Bartfai T, Yamaguchi M, Hokfelt T, Ogren SO. Microdialysis in freely moving mice: determination of acetylcholine, serotonin and noradrenaline release in galanin transgenic mice. J Neurosci Methods. 2001; 109:71–80. [PubMed: 11489302]

Kogan JH, Nestler EJ, Aghajanian GK. Elevated basal firing rates and enhanced responses to 8-Br-cAMP in locus coeruleus neurons in brain slices from opiate-dependent rats. Eur J Pharmacol. 1992; 211:47–53. [PubMed: 1618268]

Kolakowski LF Jr, et al. Molecular characterization and expression of cloned human galanin receptors GALR2 and GALR3. J Neurochem. 1998; 71:2239–2251. [PubMed: 9832121]

Koob GF, Le MM. Review. Neurobiological mechanisms for opponent motivational processes in addiction. Philos Trans R Soc Lond B Biol Sci. 2008; 363:3113–3123. [PubMed: 18653439]

Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010; 35:217–238. [PubMed: 19710631]

Kuteeva E, Hokfelt T, Ogren SO. Behavioural characterisation of transgenic mice overexpressing galanin under the PDGF-B promoter. Neuropeptides. 2005; 39:299–304. [PubMed: 15944026]

McClung CA, Nestler EJ, Zachariou V. Regulation of gene expression by chronic morphine and morphine withdrawal in the locus ceruleus and ventral tegmental area. J Neurosci. 2005; 25:6005–6015. [PubMed: 15976090]

Melander T, Hokfelt T, Rokaeus A. Distribution of galaninlike immunoreactivity in the rat central nervous system. J Comp Neurol. 1986; 248:475–517. [PubMed: 2424949]

Mennicken F, Hoffert C, Pelletier M, Ahmad S, O'donnell D. Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. J Chem Neuroanat. 2002; 24:257–268. [PubMed: 12406501]

Narasimhaiah R, Kamens HM, Picciotto MR. Effects of galanin on cocaine-mediated conditioned place preference and ERK signaling in mice. Psychopharmacology (Berl). 2009; 204:95–102. [PubMed: 19099295]

O'donnell D, Ahmad S, Wahlestedt C, Walker P. Expression of the novel galanin receptor subtype GALR2 in the adult rat CNS: distinct distribution from GALR1. J Comp Neurol. 1999; 409:469–481. [PubMed: 10379831]

Picciotto MR. Galanin and addiction. Cell Mol Life Sci. 2008; 65:1872–1879. [PubMed: 18500649]

Picciotto MR, Brabant C, Einstein EB, Kamens HM, Neugebauer NM. Effects of galanin on monoaminergic systems and HPA axis: Potential mechanisms underlying the effects of galanin on addiction- and stress-related behaviors. Brain Res. 2010; 1314:206–218. [PubMed: 19699187]

Pieribone VA, Xu ZQ, Zhang X, Grillner S, Bartfai T, Hokfelt T. Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. Neuroscience. 1995; 64:861–874. [PubMed: 7538638]

Rasmussen K, Beitner-Johnson DB, Krystal JH, Aghajanian GK, Nestler EJ. Opiate withdrawal and the rat locus coeruleus: behavioral, electrophysiological, and biochemical correlates. J Neurosci. 1990; 10:2308–2317. [PubMed: 2115910]

Seutin V, Verbanck P, Massotte L, Dresse A. Galanin decreases the activity of locus coeruleus neurons in vitro. Eur J Pharmacol. 1989; 164:373–376. [PubMed: 2474450]

Sevcik J, Finta EP, Illes P. Galanin receptors inhibit the spontaneous firing of locus coeruleus neurones and interact with mu-opioid receptors. Eur J Pharmacol. 1993; 230:223–230. [PubMed: 7678551]

Skofitsch G, Sills MA, Jacobowitz DM. Autoradiographic distribution of 125I-galanin binding sites in the rat central nervous system. Peptides. 1986; 7:1029–1042. [PubMed: 2436195]

Smith KE, Walker MW, Artymyshyn R, Bard J, Borowsky B, Tamm JA, Yao WJ, Vaysse PJ, Branchek TA, Gerald C, Jones KA. Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels. J Biol Chem. 1998; 273:23321–23326. [PubMed: 9722565]

Wang S, He C, Maguire MT, Clemmons AL, Burrier RE, Guzzi MF, Strader CD, Parker EM, Bayne ML. Genomic organization and functional characterization of the mouse GalR1 galanin receptor. FEBS Lett. 1997; 411:225–230. [PubMed: 9271210]

Xu ZQ, Bartfai T, Langel U, Hokfelt T. Effects of three galanin analogs on the outward current evoked by galanin in locus coeruleus. Ann N Y Acad Sci. 1998; 863:459–465. [PubMed: 9928199]

Zachariou V, Brunzell DH, Hawes J, Stedman DR, Bartfai T, Steiner RA, Wynick D, Langel U, Picciotto MR. The neuropeptide galanin modulates behavioral and neurochemical signs of opiate withdrawal. Proc Natl Acad Sci U S A. 2003; 100:9028–9033. [PubMed: 12853567]

Zachariou V, Georgescu D, Kansal L, Merriam P, Picciotto MR. Galanin receptor 1 gene expression is regulated by cyclic AMP through a CREB-dependent mechanism. J Neurochem. 2001; 76:191–200. [PubMed: 11145992]

Zachariou V, Parikh K, Picciotto MR. Centrally administered galanin blocks morphine place preference in the mouse. Brain Res. 1999; 831:33–42. [PubMed: 10411981]

Zachariou V, Thome J, Parikh K, Picciotto MR. Upregulation of galanin binding sites and GalR1 mRNA levels in the mouse locus coeruleus following chronic morphine treatments and precipitated morphine withdrawal. Neuropsychopharmacology. 2000; 23:127–137. [PubMed: 10882839]

Zambrowicz BP, et al. Wnk1 kinase deficiency lowers blood pressure in mice: a gene-trap screen to identify potential targets for therapeutic intervention. Proc Natl Acad Sci U S A. 2003; 100:14109–14114. [PubMed: 14610273]

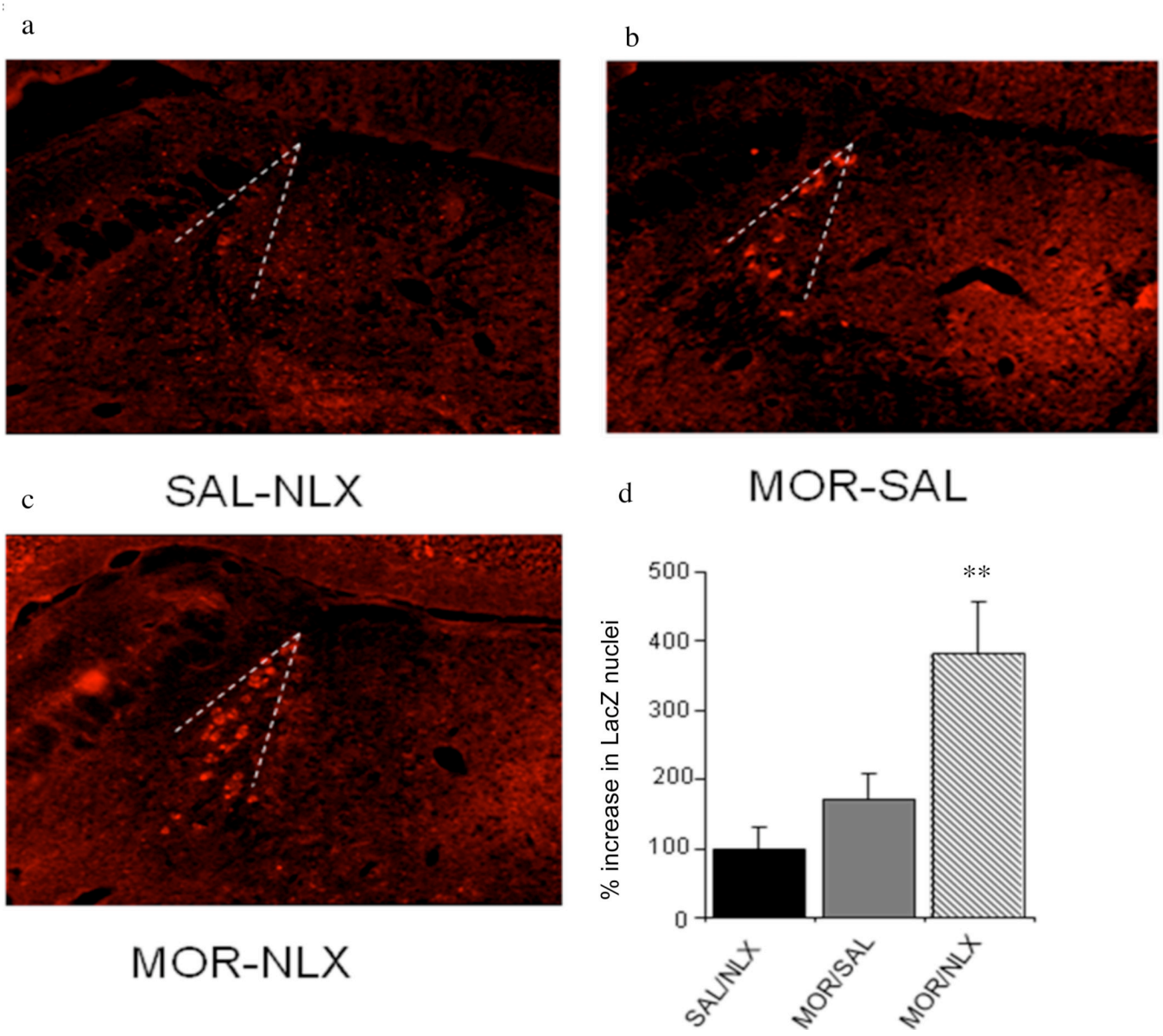

Fig. 1. Regulation of galanin promoter activity by chronic morphine and morphine withdrawal

Transgenic mice expressing beta-galactosidase (LacZ) under the control of the galanin promoter were injected with increasing doses of morphine for 3 days, as described in the Methods, and withdrawal was precipitated using naloxone. Animals received saline (a) or morphine (b and c) and on the third day received a saline (b) or naloxone (a, c) injection 3 hrs after the last morphine injection to precipitate withdrawal. Quantitation of the LacZ positive neurons in the LC following saline-naloxone (SAL-NLX), morphine (MOR), and morphine-naloxone (MOR-NLX) treatment (d) there was a significant increase in the number of LacZ positive nuclei after morphine-naloxone treatment (n=4–5 per group, **p<0.01, one way ANOVA followed by Dunnett post hoc test. LacZ expression in the LC was monitored using immunofluorescence. Micrographs were taken at 20X.

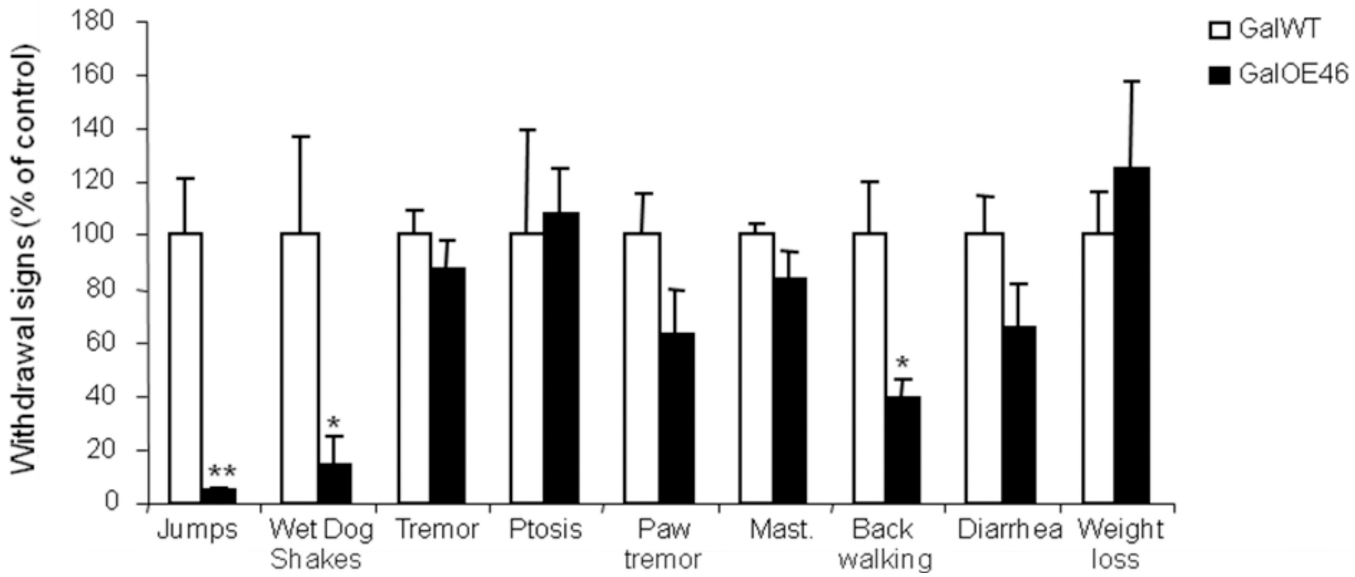

Fig. 2. Galanin overexpression in the locus coeruleus leads to a milder withdrawal syndrome

Mice over-expressing galanin (GalOE) and their wild-type (WT) controls (derived from the homozygous offspring of heterozygous breeding pairs) received increasing doses of morphine for 3 days, as described in the Methods, and withdrawal was precipitated using naloxone. Withdrawal signs (Jumps, wet dog shakes, tremor, ptosis, paw tremor, mastication (Mast.), backward walking, diarrhea and weight loss) were monitored for 30 min after naloxone injection. Data are expressed as mean ± SEM (n = 8/group). *$p < 0.05$, **$p < 0.01$, two way ANOVA followed by Bonferroni correction for multiple comparisons.

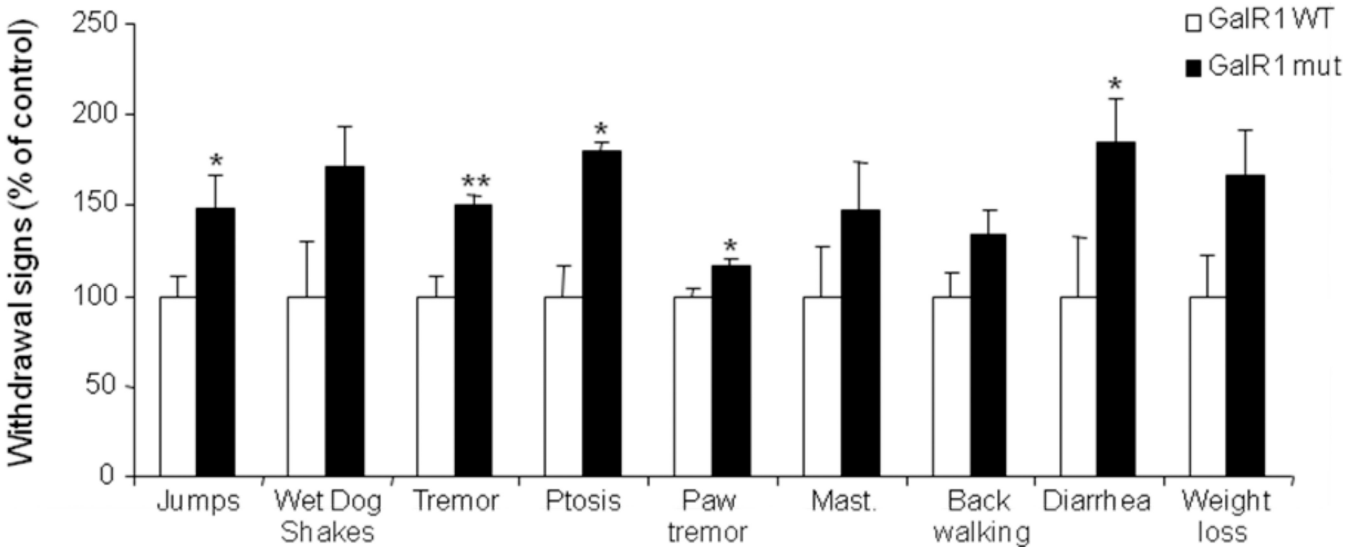

Fig. 3. GalR1 modulates morphine withdrawal behaviors

GalR1 mutant mice (GalR1 mut) and their wild-type (WT) controls (derived from the homozygous offspring of heterozygous breeding pairs) were injected with increasing doses of morphine for 3 days, as described in the Methods, and withdrawal was precipitated using naloxone. Withdrawal signs were monitored for 30 min after naloxone injection. Knockout of the GalR1 gene significantly exacerbated several signs of morphine withdrawal, including jumps, tremor, ptosis, paw tremor and diarrhea. Data are expressed as mean ± SEM (n = 7 per group). *$p < 0.05$, **$p<0.01$, two way ANOVA followed by Bonferroni correction for multiple comparisons.

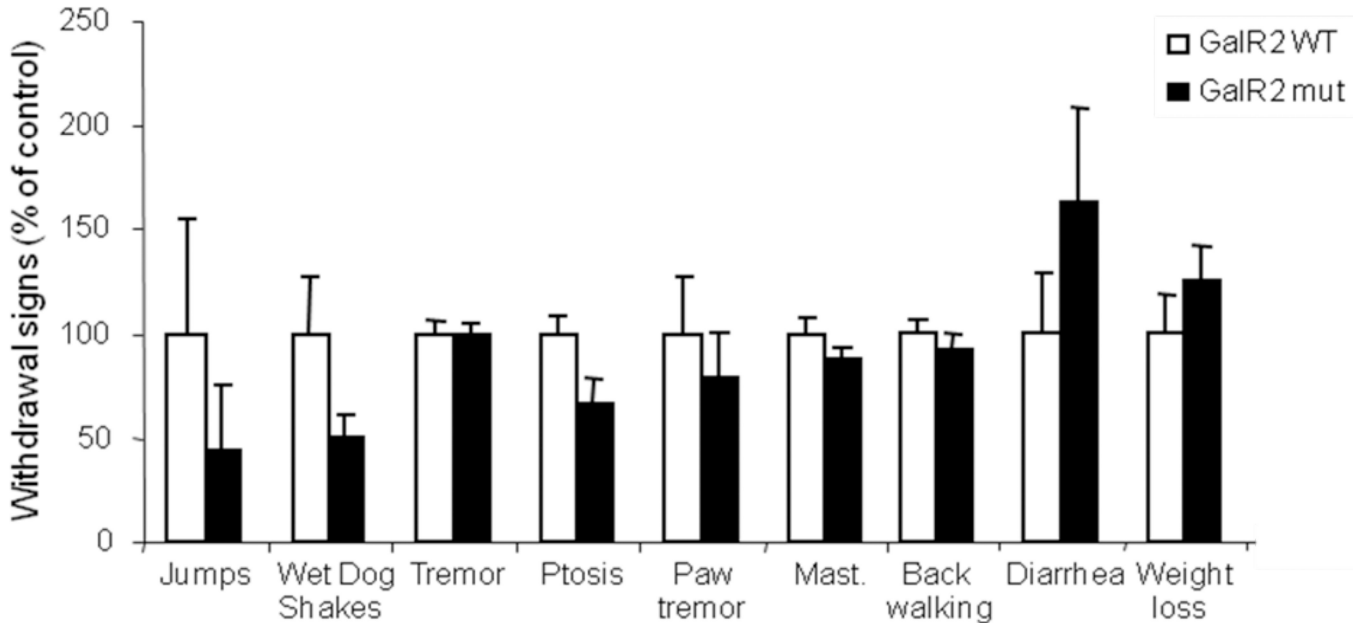

Fig. 4. Absence of GalR2 does not affect opiate withdrawal behavior

GalR2 mutant mice (GalR2 mut) and their wild-type controls (GalR2 WT) (derived from the homozygous offspring of heterozygous breeding pairs) were injected with increasing doses of morphine for 3 days, as described in the Methods, and withdrawal was precipitated using naloxone. Withdrawal signs were monitored for 30 min after naloxone injection. Lack of the GalR2 gene had no significant effect on morphine withdrawal signs. Data are expressed as mean ± SEM (n=8 per group).

Fig. 5. Absence of GalR1 or GalR2 does not affect the initial locomotor activating actions of morphine

GalR1 and GalR2 mutant mice (GalR1 mut, GalR2 mut) and their respective matched wild-type controls (WT) were injected with morphine and their locomotion was measured over thirty minutes. All mice showed comparable beam breaks regardless of genotype. Data are expressed as a percent of average saline beam breaks ± SEM.