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Galanin and addiction

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Abstract. There has been increasing interest in the ability of neuropeptides involved in feeding to modulate circuits important for responses to drugs of abuse. A number of peptides with effects on hypothalamic function also modulate the mesolimbic dopamine system (ventral tegmental area and nucleus accumbens). Similarly, common stress-related pathways can modulate food intake, drug reward and symptoms of drug withdrawal. Galanin promotes food intake and the analgesic properties of opiates; thus it initially seemed possible that galanin might potentiate opiate reinforcement. Instead, galanin agonists decrease opiate reward, measured by conditioned place preference, and opiate withdrawal signs, whereas opiate reward and withdrawal are increased in knockout mice lacking galanin. This is consistent with studies showing that galanin decreases activity-evoked dopamine release in striatal slices and decreases the firing rate of noradrenergic neurons in locus coeruleus, areas involved in drug reward and withdrawal, respectively. These data suggest that polymorphisms in genes encoding galanin or galanin receptors might be associated with susceptibility to opiate abuse. Further, galanin receptors might be potential targets for development of novel treatments for addiction. (Part of a Multi-author Review)

Keywords. Reinforcement, drug abuse, morphine, dopamine, withdrawal, norepinephrine.

Introduction

An emerging body of work has shown that neuropeptides that affect feeding can also affect drug reward. For example, cocaine- and amphetamine-regulated transcript (CART), corticotropin releasing factor (CRF), melanocortins and neuropeptide Y (NPY) all have reciprocal effects on feeding and drug reward, such that CART, leptin and CRF decrease feeding and promote drug reinforcement, whereas melanocortins and NPY increase feeding and oppose drug reward [1–7]. However, ghrelin and opiates both increase feeding and are rewarding [6, 8], suggesting that a simple reciprocal relationship cannot explain neuropeptide effects on feeding and drug reinforcement.

One site at which the interaction between feeding and drug reward occurs is thought to be the mesolimbic dopamine (DA) system. A number of recent studies have shown that neuropeptides that regulate food intake can regulate the function of the DA system. For example, leptin, the most critical peptide mediating feedback on metabolic state from peripheral tissues to the hypothalamus, also stimulates DA neuron function [7, 9]. A number of hypothalamic peptides (and their receptors) have been shown to be regulated by administration of drugs of abuse, including galanin, CART and NPY [1–4]. Thus, the ability of galanin to modulate both feeding and drug reward suggests that the coordinated regulation of appetite and hedonic aspects of food and drug intake may be co-regulated by this peptide.

Behavioral studies: effects of galanin on opiate locomotion and reward

One of the hallmarks of drugs that are abused by human beings is that they increase locomotor activity in rodents in a dopamine-dependent manner [10]. Thus, locomotor activation can provide a behavioral measure of sensitivity to drugs of abuse. Interestingly, knockout mice lacking the galanin peptide show increased locomotor responses to morphine across a

number of doses compared to their wild-type controls [11]. Galnon is a non-peptide galanin receptor agonist that can cross the blood-brain barrier [12, 13]. This compound has been useful for determining whether behavioral changes in galanin knockout mice are due to adaptation to the loss of the peptide during development or acute effects of galanin in the adult behaving animal. Galnon administration was able to reverse the morphine-induced increase in locomotor activation seen in galanin knockout mice, suggesting that even in the absence of galanin signaling throughout development, replacement of galanin action in adulthood could normalize morphine-induced locomotor activation [11].

Conditioned place preference is a contextual task that is thought to measure drug reward and drug seeking [14]. Local administration of galanin into the cerebral ventricle attenuates the rewarding effect of morphine in the mouse as measured by the place preference paradigm by shifting the dose response curve such that threshold doses are no longer rewarding in animals receiving galanin [1]. Consistent with the possibility that galanin signaling opposes opiate reward, galanin knockout mice showed increased sensitivity to a low dose of morphine in the place preference paradigm compared to their wild-type controls [11]. The effect of galanin on morphine-induced locomotion and morphine place preference occurred at very different doses, since at the threshold dose for morphine place preference (0.25 mg/kg), no locomotor activation was seen.

Regulation of the dopaminergic and cholinergic systems by galanin

Three G-protein-coupled receptors for galanin have been cloned [15–18]. GalR1 and GalR3 are generally thought to be coupled to G_i proteins, and activation of these receptors leads to inhibition of adenylyl cyclase [19], whereas GalR2 can be coupled to phospholipase C and can activate protein kinase C in neurons [19–21]. Similarly, there are three receptors for endogenous opioids, μ, δ and κ, which, like GalR1, are coupled to G_i proteins and inhibit adenylyl cyclase [22]. Knockout mouse studies show that the μ-opioid receptor is critical for reinforcing effects of morphine, as well as opiate withdrawal [23]. Since galanin can attenuate the rewarding and withdrawal actions of morphine, galanin-morphine interactions may involve the μ receptor subtype.

GalR1 mRNA is expressed at high levels in the amygdala, ventral hippocampus, thalamus and dorsal horn of the spinal cord, and in moderate amounts in the striatum, locus coeruleus (LC) and periaqueductal grey (PAG) [24]. GalR2 mRNA is expressed in the spinal cord, hippocampus, piriform and entorhinal cortex, basal nucleus of the accessory olfactory tract, amygdala, hypothalamic nuclei, ventral tegmental area (VTA), PAG and LC [16, 20, 25, 26]. GalR3 mRNA is expressed in human testis, adrenal gland and pancreas [20], with low levels in the hypothalamus and pituitary gland [27]. High levels of galanin binding sites have been measured in the dopaminergic regions of the brain [28]; however, the receptor subtypes mediating that binding cannot be identified conclusively, since the antisera raised against the GalR1 and GalR2 subunits still recognize bands on Western blots of tissue from galanin receptor knockout (GalR-KO) mouse lines [29]. Opioid receptor subtypes are also widely expressed in the brain, although μ-opioid receptors in the VTA, amygdala and LC are thought to be most critical for the rewarding effects of opiates [30].

Local administration studies into the VTA, NAc and prefrontal cortex (PFC) suggest that opiate reinforcement is mediated through activation of the mesolimbic DA system [31], but it is clear that other neurotransmitters, for example norepinephrine (NE) [32] and acetylcholine (ACh) [33, 34], can contribute to opiate reward. Acute morphine administration increases locomotor activity and can result in conditioned place preference [35, 36]. These behaviors are associated with DA release from neurons that originate in the VTA, have terminals in the nucleus accumbens (NAc), PFC, hippocampus and amygdala [35, 37], and can be blocked by both noradrenergic depletion and cholinergic receptor blockers [32–34]. Although activation of the VTA-NAc pathway results in increased locomotor activity and drug self-administration, lesions of the NAc alone are not sufficient to abolish morphine self-administration or morphine-induced locomotor activity completely [38, 39]. Thus, the NAc is one important mediator of the reinforcing effects of opiates, but other brain regions, such as the nucleus of the tractus solitarius (NTS) [32] or dorsal hippocampus [34], are also involved in opiate-induced locomotion and place preference.

The amygdala and hippocampus receive dopaminergic, noradrenergic and cholinergic projections, and are thought to participate in the reinforcing effects of drugs of abuse (for review see [40]). In addition to the VTA, NAc and PFC, animals also self-administer opiates into the dorsal hippocampus, central gray and lateral hypothalamus [41]. Thus, galanin-opiate interactions could take place in any of these brain structures. The amygdala also plays a role in morphine-induced locomotor activation [42], and amygdala DA receptor blockade can block morphine-induced locomotor activity in the rat [37, 43]. Finally,

DAergic, GABAergic, noradrenergic and cholinergic agents can all modulate morphine CPP [32, 43–45], implicating multiple systems in opiate reinforcement. As noted above, galanin receptor mRNAs [46] and galanin binding sites [28] are expressed in neurons in the mesolimbic DA system. In striatum, GalR1 mRNA is expressed in a pattern that suggests that it is localized in cholinergic interneurons [47, 48], but specific galanin binding sites are much more widespread [28], suggesting that galanin receptors may be represented on neuronal terminals in the striatum and NAc. Consistent with the ability of galanin to decrease morphine place preference, galanin has an inhibitory effect on DA release in rat striatal slices [49]. Further, administration of galanin into the VTA but not the NAc increases DOPA accumulation (suggesting a decrease in DA synthesis) throughout the striatum, and decreases spontaneous locomotor activity [50]. It is not clear, however, whether the ability of galanin to modulate DA occurs because of direct effects on DA neurons, or indirectly through modulation of other neurotransmitters.

Both galanin and morphine can modulate ACh release in the striatum/NAc, and the effects appear to be somewhat complementary. Chronic morphine increases extracellular levels of ACh in the NAc [51], and knockout of the M5 muscarinic ACh receptor decreases morphine CPP [52]. By contrast, galanin inhibits spontaneous and potassium-stimulated ACh release from both cortical slices and synaptosomes through a G_i-mediated mechanism [53]. Galanin also decreases ACh release in microdialysis studies in awake rats [54], although it can increase ACh release in striatum of anesthetized rats [54, 55], suggesting that this effect is modulated by arousal. Accordingly, galanin also decreases glutamate, but not GABA, release in striatal slices [56]. These data suggest that galanin normally decreases ACh and glutamate release in the striatum. Unfortunately, very little data is available on the effects of galanin on the VTA, but it is plausible that similar effects occur in this brain area. For example, local infusion of galanin into the VTA but not the NAc decreases locomotor activity and modulates DA synthesis [50]. Thus, effects on cholinergic neurons in the VTA and/or the NAc could underlie galanin-opiate interactions in the mesolimbic system.

Galanin-mediated modulation of intracellular signaling pathways following morphine administration

Intracellular signaling downstream of DA release is important for both locomotor activating and rewarding effects of opiates. DA release in the NAc following treatment with drugs of abuse stimulates phosphorylation of extracellular-signaling regulated protein kinase (ERK) leading to increased activity of this protein, and this is thought to be important for locomotor activation and reward [57]. Indeed, inhibition of ERK phosphorylation in the VTA blocks the rewarding effects of morphine [58]. Morphine induces a significant increase in the activity of ERK in the VTA, NAc and amygdala of both galanin wild-type and knockout animals, but the increase is significantly greater in knockout mice lacking the galanin peptide [11]. In the VTA, galnon administration decreased morphine-induced ERK phosphorylation in both wild-type and galanin knockout mice, suggesting that activation of galanin receptors in the VTA can attenuate activity of DA neurons and decrease signaling related to opiate reward; however, the decrease in P-ERK by galnon was not complete, suggesting that morphine also regulates ERK activity independent of the galanin system. In the NAc and amygdala, galnon decreased ERK phosphorylation back to baseline levels in galanin knockout mice, but had no effect on the already low baseline in wild-type mice. Taken together, these data demonstrate that the consequences of morphine administration on intracellular signaling in the mesolimbic DA system are exacerbated in the absence of galanin and attenuated by the administration of a galanin agonist.

Behavioral responses to drugs of abuse depend on persistent alterations in neuronal activity that occur following induction of a number of intracellular signaling cascades [59]. The ability of drugs of abuse to recruit signaling pathways in DA neurons and their downstream targets in the NAc, amygdala and PFC is critical for neuronal plasticity leading to persistent behavioral changes [60–62]. Thus, the ability of galanin and morphine to modulate similar intracellular signaling cascades may underlie galanin-opiate interactions at the behavioral level. As mentioned above, both GalR1 and the μ-opioid receptor are coupled to G_i proteins and both can decrease cyclic AMP (cAMP) levels, as well as downstream signaling through cAMP-dependent protein kinase (PKA) [22, 63]. In the VTA, μ-opioid receptors are thought to act predominantly on GABAergic neurons [64]. By contrast, GalR1 message may be found in cholinergic neurons [46, 48]. Thus, morphine, acting through μ-opioid receptors, decreases activity of the cAMP pathway in GABA neurons thereby disinhibiting DA cell bodies and terminals [65]. By contrast, the effects of galanin on this circuit are still relatively unknown, but galanin can decrease ACh release [53], as well as locomotor activity through actions in the VTA [50]. Thus, in a highly speculative model (Fig. 1), galanin

could decrease either DA release in the VTA directly or indirectly through decrease of ACh release, leading to decreased cholinergic inputs to DA neuron cell bodies. Therefore, circuit-level interactions between galanin and opiates in the VTA or NAc could have opposing effects on output of this system.

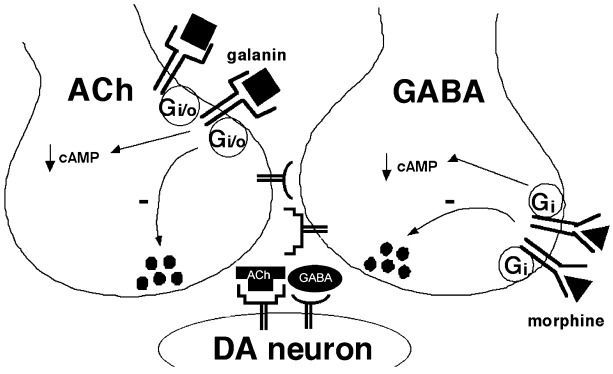

Figure 1. Model for galanin-opiate interactions at the cellular level. GalR1 and μ-opioid receptors decrease both cAMP levels and neuronal excitability. Thus, in cells co-expressing both receptor subtypes (not shown), galanin could substitute for morphine, perhaps explaining the ability of galanin to counteract some somatic signs of opiate withdrawal. By contrast, in brain regions in which GalRs and opioid receptors are on different neuronal subtypes, galanin and morphine could have opposing actions. In the hypothetical case depicted here, μ-opioid receptors on GABA neurons result in decreased GABA release onto DA neurons, disinhibiting these neurons. Galanin results in decreased ACh release, potentially decreasing cholinergic input to DA neurons from the pedunculopontine tegmental area, an effect known to decrease opiate reward [87, 88]. Connections between GABA and ACh neurons would amplify this interaction [87]. The interaction is depicted on the terminals here since galanin can modulate release of neurotransmitters in slice preparations, but the effects of both galanin and opiates would likely occur on cell bodies as well. DA, dopamine; ACh, acetylcholine.

Galanin and alcohol

Alcohol, like other drugs abused by humans, increases extracellular DA levels in the NAc [66]. A major difference between alcohol and other drugs, however, is that alcohol has caloric content. Thus, peptides that regulate feeding as well as responses to drugs of abuse could have extremely complex effects on alcohol intake. Both pharmacological and genetic studies have shown that galanin normally decreases opiate and cocaine reward [1, 11, 67]. In contrast, galanin increases intake of ethanol [68–70], increases hypothalamic expression of the mRNA encoding galanin [71, 72] and potentially decreases GalR1 [73]. The ability of galanin signaling to regulate alcohol intake is especially interesting given that single nucleotide polymorphisms in the galanin gene and the GalR gene have been associated with alcoholism [74, 75]. Ongoing studies should determine whether the effects of galanin on alcohol intake are mediated through similar or distinct mechanisms to regulation of food intake and preference for other drugs of abuse.

Galanin and opiate withdrawal: behavioral studies

In addition to effects on opiate reward, galanin also has effects on the consequences of chronic opiate administration. Systemic administration of the galanin agonist galnon significantly attenuates several opiate withdrawal signs in mice [63]. In addition, activation of galanin receptors by endogenous galanin normally plays a protective role against opiate dependence and withdrawal since knockout mice lacking the galanin gene show significantly more withdrawal signs than wild-type animals, and this increase can be reversed by galnon as well. It should be noted that one report showed that ventricular administration of galanin failed to suppress somatic opiate withdrawal symptoms at doses that induced feeding [76], perhaps because the peptide did not penetrate fully through the brain stem. The noradrenergic system is an important site of action for galnon or endogenous galanin in attenuating opiate withdrawal since transgenic mice expressing galanin under the control of a promoter that targets expression to noradrenergic neurons [77] show significant decreases in several withdrawal signs as compared to their wild-type siblings. These data suggest that galanin's effects on opiate withdrawal signs may be mediated through actions on the noradrenergic system.

Potential mechanisms underlying effects of galanin on morphine withdrawal

Lesion of the LC, the major noradrenergic nucleus in the brain, reduces a subset of opiate withdrawal symptoms, suggesting that the LC is one brain area that contributes to the expression of some physical signs of opiate dependence [78]. Many studies of galanin action have focused on modulation of physiological properties of LC neurons. Galanin is found at high levels in most norepinephrine-positive neurons of the LC and can decrease the firing rate of LC neurons in brain slices [79–81]. Based on transgenic mouse studies in which galanin is overexpressed in noradrenergic neurons [63] as well as physiological studies [80], a possible mechanism underlying galnon antagonism of opiate withdrawal signs could be decreased firing of noradrenergic neurons. Consistent with this possibility, expression of c-fos, a marker of neuronal activation, is greatly increased in the LC

during opiate withdrawal, and this increase is attenuated following administration of galnon [63].
One potential mechanism underlying the decreased firing rate of LC neurons reflected in c-fos measurements is a decrease in cAMP levels as a result of activation of galanin receptors coupled to G_i proteins. It has been shown previously that serine 40 in TH is specifically phosphorylated by PKA. Consistent with this possibility, tyrosine hydroxylase phosphorylation on a PKA-sensitive site, a measure of cellular cAMP levels [82] in catecholaminergic neurons, is significantly increased following morphine withdrawal, and this increase is greatly reduced by galnon administration.

Galanin-mediated biochemical changes following opiate withdrawal

The hypothesis that the mechanism underlying effects of galanin on opiate withdrawal might involve inhibition of cAMP-dependent signaling is supported by *in vitro* studies. In Cath.a cells, an immortalized LC-like cell line, as well as in primary striatal neurons, chronic morphine, followed by naloxone challenge to simulate opiate withdrawal, increases PKA-mediated phosphorylation of target proteins, and this is attenuated by galanin administration [83].
There is also plasticity in the galanin system following opiate administration and withdrawal that modulates response to endogenous galanin. Galanin binding and gene expression of GalR1 are increased in the LC during opiate withdrawal [48]. Therefore, galanin receptor regulation may play an important role in galanin-influenced physiological functions of noradrenergic neurons. GalR1 contains promoter elements that are responsive to the cAMP-sensitive transcription factor CREB [84]. GalR1 is upregulated in a cAMP/CREB-dependent manner as part of a negative feedback mechanism that does not extend to GalR2 and GalR3 [85]. These observations suggest that GalR1, but not GalR2 or GalR3, can be regulated by events that alter cAMP-dependent signaling pathways in noradrenergic neurons, such as opiate withdrawal.

Conclusions

In neurons expressing both GalR1 and the μ-opioid receptor there are likely to be similar consequences on the cAMP pathway following stimulation with galanin or morphine, such that both peptides would decrease cAMP levels and decrease the firing rate of these neurons. This may explain why galanin can oppose opiate withdrawal. One hypothesis for the molecular basis of opiate withdrawal is that chronic opiate receptor activation, and the attendant activation of G_i and decrease in cAMP levels, leads to compensatory increases in adenylyl cyclase and PKA activity, such that activity is normalized when the opiate is onboard [86]. This hypothesis goes on to suggest that opiate withdrawal relieves the tonic inhibition on the cAMP system, resulting in greatly increased PKA activity and increased firing of noradrenergic neurons by decreasing a hyperpolarizing potassium current [22]. Since GalR1 is also coupled to G_i proteins, galanin compensates for loss of opiate stimulation during withdrawal and normalizes cAMP levels [63] and firing rate [79].
While galanin substitutes for morphine in brain areas involved in withdrawal, it is not rewarding on its own [1], suggesting that the effects of galanin on opiate withdrawal and reward are distinct. This is consistent with the idea that opiate withdrawal and reward are mediated through different brain regions, and that in brain areas in which μ-opioid receptors and GalR1 are on different neuronal subtypes, the similar coupling to downstream signaling pathways results in opposing actions on neuronal excitability in the circuit (Fig. 1). The studies reviewed here support the idea that galanin normally acts to counteract opiate reward and withdrawal, and that small-molecule GalR agonists can diminish opiate reward and signs of withdrawal. Many questions remain to be answered, however. The effects of galanin on alcohol intake are opposite to those on cocaine and morphine seeking, perhaps because of the caloric content of alcohol. Further studies are necessary to identify the mechanisms underlying the two sets of effects. In addition, while the effects of galanin on drug reward are likely to be due to actions on the mesolimbic system, it is not yet known whether the effects of galanin on DA neurons are direct or indirect. The cholinergic system is an attractive target for future studies of galanin-mediated modulation of DA signaling. Despite these open questions, GalRs may be attractive targets for the development of novel therapeutics for drug addiction.

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1 Zachariou, V., Parikh, K. and Picciotto, M. R. (1999) Centrally administered galanin blocks morphine place preference in the mouse. Brain Res. 831, 33–42.
2 Li, M. D., Kane, J. K., Parker, S. L., McAllen, K., Matta, S. G. and Sharp, B. M. (2000) Nicotine administration enhances NPY expression in the rat hypothalamus. Brain Res. 867, 157–164.

3 Hunter, R. G. and Kuhar, M. J. (2003) CART peptides as targets for CNS drug development. Current Drug Targets CNs Neurol. Disord. 2, 201–205.
4 Alvaro, J. D., Tatro, J. B., Quillan, J. M., Fogliano, M., Eisenhard, M., Lerner, M. R., Nestler, E. J. and Duman, R. S. (1996) Morphine down-regulates melanocortin-4 receptor expression in brain regions that mediate opiate addiction. Mol. Pharmacol. 50, 583–591.
5 Gilpin, N. W., Stewart, R. B., Murphy, J. M., Li, T. K. and Badia-Elder, N. E. (2003) Neuropeptide Y reduces oral ethanol intake in alcohol-preferring (P) rats following a period of imposed ethanol abstinence. Alcohol. Clin. Exper. Res. 27, 787–794.
6 Yeomans, M. R. and Gray, R. W. (2002) Opioid peptides and the control of human ingestive behaviour. Neurosci. Biobehav. Rev. 26, 713–728.
7 Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., Maratos-Flier, E. and Flier, J. S. (2006) Leptin regulation of the mesoaccumbens dopamine pathway. Neuron 51, 811–822.
8 Abizaid, A., Liu, Z.-W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., Roth, R. H., Sleeman, M. W., Picciotto, M. R., Tschop, M. H. et al. (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J. Clin. Invest. 116, 3229–3239.
9 Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., Thurmon, J. J., Marinelli, M. and DiLeone, R. J. (2006) Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 51, 801–810.
10 Wise, R. A. and Bozarth, M. A. (1987) A psychomotor stimulant theory of addiction. Psycholog. Rev. 94, 469–492.
11 Hawes, J. J., Brunzell, D. H., Narasimhaiah, R., Langel, U., Wynick,D. and Picciotto,M.R. (2007) Galanin protects against behavioral and neurochemical correlates of opiate reward. Neuropsychopharmacology, advance online publication, October 24, 2007; doi:10.1038/sj.npp.1301579.
12 Saar, K., Mazarati, A. M., Mahlapuu, R., Hallnemo, G., Soomets, U., Kilk, K., Hellberg, S., Pooga, M., Tolf, B.-R., Shi, T. S. et al. (2002) Anticonvulsant activity of a nonpeptide galanin receptor agonist. Proc. Natl. Acad. Sci., USA 99, 7136–7141.
13 Wu, W. P., Hao, J. X., Lundstrom, L., Wiesenfeld-Hallin, Z., Langel, U., Bartfai, T. and Xu, X. J. (2003) Systemic galnon, a low-molecular weight galanin receptor agonist, reduces heat hyperalgesia in rats with nerve injury. Eur. J. Pharmacol. 482, 133–137.
14 Schechter, M. D. and Calcagnetti, D. J. (1993) Trends in place preference conditioning with a cross-indexed bibliography: 1957–1991. Neurosci. Biobehav. Rev. 17, 21–41.
15 Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M. and Mayaux, J. F. (1994) Molecular cloning of a functional human galanin receptor. Proc. Natl. Acad. Sci. USA 91, 9780–9783.
16 Wang, S., Hashemi, T., He, C., Strader, C. and Bayne, M. (1997) Molecular cloning and pharmacological characterization of a new galanin receptor subtype. Mol. Pharmacol. 52, 337–343.
17 Wang, S., He, C., Hashemi, T. and Bayne, M. (1997) Cloning and expression characterization of a novel galanin receptor. J. Biol. Chem. 272, 31949–31952.
18 Pang, L., Hashemi, T., Lee, H. J., Maguire, M., Graziano, M. P., Bayne, M., Hawes, B., Wong, G. and Wang, S. (1998) The mouse GalR2 galanin receptor: genomic organization, cDNA cloning, and functional characterization. J. Neurochem. 71, 2252–2259.
19 Wang, S., Hashemi, T., Fried, S., Clemmons, A. L. and Hawes, B. E. (1998) Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes. Biochemistry 37, 6711–6717.
20 Kolakowski, L. F., Jr., O'Neill, G. P., Howard, A. D., Broussard, S. R., Sullivan, K. A., Feighner, S. D., Sawzdargo, M., Nguyen, T., Kargman, S., Shiao, L. L. et al. (1998) Molecular characterization and expression of cloned human galanin receptors GALR2 and GALR3. J. Neurochem. 71, 2239–2251.
21 Hawes, J. J., Narasimhaiah, R. and Picciotto, M. R. (2006) Galanin and galanin-related peptide modulate neurite outgrowth via PKC-mediated activation of ERK. Eur. J. Neurosci. 23, 2937–2946.
22 Aghajanian, G. K. and Wang, Y. Y. (1987) Common alpha 2- and opiate effector mechanisms in the locus coeruleus: intracellular studies in brain slices. Neuropharmacology 26, 793–799.
23 Matthes, H. W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dolle, P. et al. (1996) Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 383, 819–823.
24 Burgevin, M. C., Loquet, I., Quarteronet, D. and Habert-Ortoli, E. (1995) Cloning, pharmacological characterization, and anatomical distribution of a rat cDNA encoding for a galanin receptor. J. Mol. Neurosci. 6, 33–41.
25 Howard, A. D., Tan, C., Shiao, L. L., Palyha, O. C., McKee, K. K., Weinberg, D. H., Feighner, S. D., Cascieri, M. A., Smith, R. G., Van Der Ploeg, L. H. et al. (1997) Molecular cloning and characterization of a new receptor for galanin. FEBS Lett. 405, 285–290.
26 Depczynski, B., Nichol, K., Fathi, Z., Iismaa, T., Shine, J. and Cunningham, A. (1998) Distribution and characterization of the cell types expressing GALR2 mRNA in brain and pituitary gland. Ann. N. Y. Acad. Sci. 863, 120–128.
27 Smith, K. E., Walker, M. W., Artymyshyn, R., Bard, J., Borowsky, B., Tamm, J. A., Yao, W. J., Vaysse, P. J., Branchek, T. A., Gerald, C. et al. (1998) Cloned human and rat galanin GALR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels. J. Biol. Chem. 273, 23321–23326.
28 Hawes, J. J. and Picciotto, M. R. (2004) Characterization of GalR1, GalR2, and GalR3 immunoreactivity in catecholaminergic nuclei of the mouse brain. J. Comp. Neurol. 479, 410–423.
29 Hawes, J. J. and Picciotto, M. R. (2005) GalR1 and GalR2 antisera recognize bands in their respective knockout mice (erratum for JCN 479:410) J. Comp. Neurol. 490, 98–100.
30 Wise, R. (1989) Opiate reward: sites and substrates. Neurosci. Biobehav. Rev. 13, 129–133.
31 Koob, G. F., Maldonado, R. and Stinus, L. (1992) Neural substrates of opiate withdrawal. Trends Neurosci. 15, 186–191.
32 Olson, V. G., Heusner, C. L., Bland, R. J., During, M. J., Weinshenker, D. and Palmiter, R. D. (2006) Role of noradrenergic signaling by the nucleus tractus solitarius in mediating opiate reward. Science 311, 1017–1020.
33 Hikida, T., Kitabatake, Y., Pastan, I. and Nakanishi, S. (2003) Acetylcholine enhancement in the nucleus accumbens prevents addictive behaviors of cocaine and morphine Proc. Natl. Acad. Sci. USA 100, 6169–6173.
34 Rezayof, A., Zatali, H., Haeri-Rohani, A. and Zarrindast, M.-R. (2006) Dorsal hippocampal muscarinic and nicotinic receptors are involved in mediating morphine reward. Behav. Brain Res. 166, 281–290.
35 Wise, R. (1987) The role of reward pathways in the development of drug dependence. Pharm. Ther. 35, 227–263.
36 Suzuki, T., Funada, M., Narita, M., Misawa, M. and Nagase, H. (1993) Morphine-induced place preference in the CXBK mouse: characteristics of mu opioid receptor subtypes. Brain Res. 602, 45–52.
37 Chang, C., Wang, N. and Lin, M. (2004) Inhibition of the dopamine system in rat amygdala attenuates the picrotoxin-induced locomotor hyperactivity and hypertension. Clin. Exp. Pharmacol. Physiol.
38 Bunney, W., Massar, I. V. and Pert, A. (1984) Chronic morphine-induced hyperactivity in rats is altered by nucleus accumbens and ventral tegmental lesions. Psychopharmacology 82, 318–321.

39 Gerrits, M. and Van Ree, J. (1996) Effect of nucleus accumbens dopamine depletion on motivational aspects involved in initiation of cocaine and heroin self-administration in rats. Brain Res. 713, 114–124.

40 Jentsch, J. and Taylor, J. (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control over behavior by reward-related stimuli. Psychopharmacology, 146(4):373–390.

41 McBride, W. J., Murphy, J. M. and Ikemoto, S. (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav. Brain Res. 101, 129–152.

42 Libri, V., Ammassari-Teule, M., Castellano, C., Libri, V., Ammassari-Teule, M. and Castellano, C. (1989) Blocking of morphine-induced locomotor hyperactivity by amygdaloid lesions in C57BL/6 mice. Brain Res. 479, 1–5.

43 Rezayof, A., Zarrindast, M., Sahraei, H. and Haeri-Rohani, A. (2002) Involvement of dopamine D2 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. Pharmacol. Biochem. Behav. 74, 187–197.

44 Zarrindast, M. R., Massoudi, R., Sepehri, H., Rezayof, A., Zarrindast, M.-R., Massoudi, R., Sepehri, H. and Rezayof, A. (2006) Involvement of GABA(B) receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats. Physiol. Behav. 87, 31–38.

45 Zarrindast, M. R., Fattahi, Z., Rostami, P., Rezayof, A., Zarrindast, M.-R., Fattahi, Z., Rostami, P. and Rezayof, A. (2005) Role of the cholinergic system in the rat basolateral amygdala on morphine-induced conditioned place preference. Pharm. Biochem. Behav. 82, 1–10.

46 Gustafson, E. L., Smith, K. E., Durkin, M. M., Gerald, C. and Branchek, T. A. (1996) Distribution of a rat galanin receptor mRNA in rat brain. Neuroreport 7, 953–957.

47 O'Donnell, D., Ahmad, S., Wahlestedt, C. and 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.

48 Zachariou, V., Thome, J., Parikh, K. and Picciotto, M. R. (2000) Upregulation of galanin binding sites and GalR1 mRNA levels in the mouse locus coeruleus following chronic morphine treatments and precipitated morphine withdrawal. Neuropsychopharmacology 23, 127–137.

49 Tsuda, K., Tsuda, S., Nishio, I., Masuyama, Y. and Goldstein, M. (1998) Effects of galanin on dopamine release in the central nervous system of normotensive and spontaneously hypertensive rats. Am. J. Hypertens. 11, 1475–1479.

50 Ericson, E. and Ahlenius, S. (1999) Suggestive evidence for inhibitory effects of galanin on mesolimbic dopaminergic neurotransmission. Brain Res. 822, 200–209.

51 Fiserova, M., Consolo, S. and Krsiak, M. (1999) Chronic morphine induces long-lasting changes in acetylcholine release in rat nucleus accumbens core and shell: an in vivo microdialysis study. Psychopharmacology 142, 85–94.

52 Basile, A. S., Fedorova, I., Zapata, A., Liu, X., Shippenberg, T., Duttaroy, A., Yamada, M. and Wess, J. (2002) Deletion of the M5 muscarinic acetylcholine receptor attenuates morphine reinforcement and withdrawal but not morphine analgesia. Proc. Natl. Acad. Sci. USA 99, 11452–11457.

53 Wang, H. Y., Wild, K. D., Shank, R. P. and Lee, D. H. (1999) Galanin inhibits acetylcholine release from rat cerebral cortex via a pertussis toxin-sensitive G(i)protein. Neuropeptides 33, 197–205.

54 Antoniou, K., Kehr, J., Snitt, K. and Ögren, S. O. (1997) Differential effects of the neuropeptide galanin on striatal acetylcholine release in anaesthetized and awake rats. Br. J. Pharmacol. 121, 1180–1186.

55 Ögren, S. O., Pramanik, A., Land, T. and Langel, Ü. (1993) Differential effects of the putative galanin receptor antagonists M15 and M35 on striatal acetylcholine release. Eur. J. Pharmacol. 242, 59–64.

56 Ellis, Y. and Davies, J. A. (1994) The effect of neuropeptides on the release of neurotransmitter amino acids from rat striatum. Neuropeptides 26, 65–69.

57 Valjent, E., Pages, C., Herve, D., Girault, J. and Caboche, J. (2004) Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. Eur. J. Neurosci. 19, 1826–1836.

58 Ozaki, S., Narita, M., Narita, M., Ozaki, M., Khotib, J. and Suzuki, T. (2004) Role of extracellular signal-regulated kinase in the ventral tegmental area in the suppression of the morphine-induced rewarding effect in mice with sciatic nerve ligation. J. Neurochem. 88, 1389–1397.

59 Nestler, E. (2002) Common molecular and cellular substrates of addiction and memory. Neurobiol. Learn. Mem. 78, 637–647.

60 Nestler, E. J., Hope, B. T. and Widnell, K. L. (1993) Drug addiction: a model for the molecular basis of neural plasticity. Neuron 11, 995–1006.

61 Kalivas, P. W., Volkow, N. and Seamans, J. (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45, 647–650.

62 Saal, D., Dong, Y., Bonci, A. and Malenka, R. C. (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37, 577–582.

63 Zachariou, V., Brunzell, D. H., Hawes, J., Stedman, D. R., Bartfai, T., Steiner, R. A., Wynick, D., Langel, U. and Picciotto, M. R. (2003) The neuropeptide galanin modulates behavioral and neurochemical signs of opiate withdrawal. Proc. Natl. Acad. Sci. USA 100, 9028–9033.

64 Bergevin, A., Girardot, D., Bourque, M.-J. and Trudeau, L.-E. (2002) Presynaptic mu-opioid receptors regulate a late step of the secretory process in rat ventral tegmental area GABAergic neurons. Neuropharmacology 42, 1065–1078.

65 Schoffelmeer, A. N., Wardeh, G. and Vanderschuren, L. J. (2001) Morphine acutely and persistently attenuates non-vesicular GABA release in rat nucleus accumbens. Synapse 42, 87–94.

66 Weiss, F. and Porrino, L. J. (2002) Behavioral neurobiology of alcohol addiction: recent advances and challenges. J. Neurosci. 22, 3332–3337.

67 Narasimhaiah, R. and Picciotto, M. R. (2006) Galanin attenuates cocaine-conditioned place preference and ERK signaling. Soc. Neurosci. Abstr. 32.

68 Lewis, M. J., Johnson, D. F., Waldman, D., Leibowitz, S. F. and Hoebel, B. G. (2004) Galanin microinjection in the third ventricle increases voluntary ethanol intake. Alcohol. Clin. Exp. Res. 28, 1822–1828.

69 Rada, P., Avena, N. M., Leibowitz, S. F. and Hoebel, B. G. (2004) Ethanol intake is increased by injection of galanin in the paraventricular nucleus and reduced by a galanin antagonist. Alcohol 33, 91–97.

70 Schneider, E. R., Rada, P., Darby, R. D., Leibowitz, S. F. and Hoebel, B. G. (2007) Orexigenic peptides and alcohol intake: differential effects of orexin, galanin, and ghrelin. Alcoholic Clin. Exp. Res. 31, 1858–1865.

71 Leibowitz, S. F., Avena, N. M., Chang, G.-Q., Karatayev, O., Chau, D. T. and Hoebel, B. G. (2003) Ethanol intake increases galanin mRNA in the hypothalamus and withdrawal decreases it. Physiol. Behav. 79, 103–111.

72 Chang, G.-Q., Karatayev, O., Ahsan, R., Avena, N. M., Lee, C., Lewis, M. J., Hoebel, B. G. and Leibowitz, S. F. (2007) Effect of ethanol on hypothalamic opioid peptides, enkephalin, and dynorphin: relationship with circulating triglycerides Alcohol. Clin. Exp. Res. 31, 249–259.

73 Pickering, C., Avesson, L., Liljequist, S., Lindblom, J. and Schiçth, H. (2007) The role of hypothalamic peptide gene expression in alcohol self-administration behavior. Peptides 28(12), 2361–2371.

74 Belfer, I., Hipp, H., McKnight, C., Evans, C., Buzas, B., Bollettino, A., Albaugh, B., Virkkunen, M., Yuan, Q., Max, M. B. et al. (2006) Association of galanin haplotypes with alcohol-

ism and anxiety in two ethnically distinct populations. Mol. Psychiatry 11, 301–311.
75 Belfer, I., Hipp, H., Bollettino, A., McKnight, C., Evans, C., Virkkunen, M., Albaugh, B., Max, M. B., Goldman, D. and Enoch, M. A. (2007) Alcoholism is associated with GALR3 but not two other galanin receptor genes Genes. Brain Behav. 6, 473–481.
76 Holmes, P. V., Koprivica, V., Chough, E. and Crawley, J. N. (1994) Intraventricular administration of galanin does not affect behaviors associated with locus coeruleus activation in rats. Peptides 15, 1303–1308.
77 Steiner, R. A., Hohmann, J. G., Holmes, A., Wrenn, C. C., Cadd, G., Juréus, A., Clifton, D. K., Luo, M. L., Gutshall, M., Ma, S. Y. et al. (2001) Galanin transgenic mice display cognitive and neurochemical deficits characteristic of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 98, 4184–4189.
78 Maldonado, R. and Koob, G. F. (1993) Destruction of the locus coeruleus decreases physical signs of opiate withdrawal. Brain Res. 605, 128–138.
79 Pieribone, V. A., Xu, Z. Q., Zhang, X., Grillner, S., Bartfai, T. and Hökfelt, T. (1995) Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. Neuroscience 64, 861–874.
80 Sevcik, J., Finta, E. P. and 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.
81 Seutin, V., Verbanck, P., Massotte, L. and Dresse, A. (1989) Galanin decreases the activity of locus coeruleus neurons in vitro. Eur. J. Pharmacol. 164, 373–376.
82 Salvatore, M. F., Garcia-Espana, A., Goldstein, M., Deutch, A. Y. and Haycock, J. W. (2000) Stoichiometry of tyrosine hydroxylase phosphorylation in the nigrostriatal and mesolimbic systems in vivo: effects of acute haloperidol and related compounds. J. Neurochem. 75, 225–232.
83 Hawes, J. J., Narasimhaiah, R. and Picciotto, M. R. (2006) 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. 96, 1160–1168.
84 Zachariou, V., Georgescu, D., Kansal, L., Merriam, P. and Picciotto, M. R. (2001) Galanin receptor 1 gene expression is regulated by cyclic-AMP through a CREB-dependent mechanism. J. Neurochem. 76, 191–200.
85 Hawes, J. J., Brunzell, D. H., Wynick, D., Zachariou, V. and Picciotto, M. R. (2005) GalR1, but not GalR2 or GalR3, levels are regulated by galanin signaling in the locus coeruleus through a cyclic AMP-dependent mechanism. J. Neurochem. 93, 1168–1176.
86 Rasmussen, K., Beitner-Johnson, D. B., Krystal, J. H., Aghajanian, G. K. and Nestler, E. J. (1990) Opiate withdrawal and the rat locus coeruleus: behavioral, electrophysiological, and biochemical correlates. J. Neurosci. 10, 2308–2317.
87 Miller, A. D., Forster, G. L., Yeomans, J. S. and Blaha, C. D. (2005) Midbrain muscarinic receptors modulate morphine-induced accumbal and striatal dopamine efflux in the rat. Neuroscience 136, 531–538.
88 Bechara, A. and van der Kooy, D. (1989) The tegmental pedunculopontine nucleus: a brain-stem output of the limbic system critical for the conditioned place preferences produced by morphine and amphetamine. J. Neurosci. 9, 3400–3409.