Society for Endocrinology
JOURNAL OF MOLECULAR ENDOCRINOLOGY

The sigma-1 receptor as key common factor in cocaine and food seeking behaviors

Journal:	*Journal of Molecular Endocrinology*
Manuscript ID	JME-19-0138
mstype:	Invited review
Date Submitted by the Author:	02-Jul-2019
Complete List of Authors:	Aguinaga, David; CIBERNED, Parkinson Division 201 Casanovas, Mireia; Universitat de Barcelona, Bioquimica i Biomedicina Molecular Rivas.-Santisteban, Rafael; Universitat de Barcelona, Bioquimica i Biomedicina Molecular Reyes-Resina, Irene; Universitat de Barcelona, Bioquimica i Biomedicina Molecular Navarro, Gemma; Universitat de Barcelona, Bioquimica i Fisiologia Franco, Rafael; Universitat de Barcelona, Biochem Mol Biomedicine; CIBERNED, Parkinson's Division 201
Keywords:	Ghrelin, orexin, Hormone receptors, Signal transduction, Calcium signalling

The sigma-1 receptor as key common factor in cocaine and food seeking behaviors

David Aguinaga[1,2], Mireia Casanovas[1,2], Rafael Rivas-Santisteban[1,2], Irene Reyes-Resina[1,2], Gemma Navarro[2,3,*], Rafael Franco[1,2,*]

1. Dept. Biochemistry and Molecular Biomedicine. School of Biology. Universitat de Barcelona. Barcelona. Spain.
2. Centro de Investigación en Red, enfermedades Neurodegenerativas. CiberNed. Instituto de Salud Carlos III. Madrid. Spain.
3. Dept. Biochemistry and Physiology. School of Pharmacy. Universitat de Barcelona. Barcelona. Spain.

* Equal contribution

Corresponding authors:

- Rafael Franco.

Dept. Biochemistry and Molecular Biomedicine. School of Biology. Prevosti Building. Universitat de Barcelona. Diagonal 643. 08028. Barcelona. Spain.

rfranco123@gmail.com; rfranco@ub.edu

Tel +34934021208

- Gemma Navarro

Dept. Biochemistry and Physiology. School of Pharmacy. Universitat de Barcelona. 08028. Barcelona. Spain.

rfranco123@gmail.com; rfranco@ub.edu

Tel +34934034500

Short title

Cocaine, ghrelin, sigma-1 receptors, and appetite

Keywords

Appetite, cAMP, calcium signaling, MAP kinase, reward circuits, CNS.

Abstract

Addiction and eating disorders involve brain reward circuits. A previous history of binge eating predisposes to addictive behavior, while the cessation of exposure to drugs of abuse leads to reward activities, including intake of tasty foods. Cocaine use is associated with a decrease in food intake, with reversal after the drug use is stopped. Exciting new findings show that receptors for the "hunger" hormone, ghrelin, directly interact with the sigma-1 receptors (σ_1R), which is a target of cocaine. σ_1R are key players in regulating dopaminergic neurotransmission and ghrelin-mediated actions. This review focuses on the σ_1 receptor as general neuroendocrine regulator by directly interacting with neuronal G-protein-coupled receptors. This review also covers the early mechanisms by which cocaine binding to σ_1 blocks the food-seeking behavior triggered by ghrelin. Such new findings appear as fundamental to understand common mechanisms in drug addiction and eating disorders.

Introduction

Neuroendocrine mechanisms involving food seeking behavior involve peripheral and central players being ghrelin one of the most relevant. Ghrelin is synthesized in the stomach but its endocrine action is, among other, exerted in heart, gastrointestinal (GI) tract, pancreas and central nervous system (CNS) (Figure 1). The "hunger" ghrelin hormone mediates their actions via a specific receptor, GHS-R1a, which belongs to the family of G-protein-coupled receptors (GPCRs). Dopamine, one of the main neurotransmitters in the brain is involved in the circuits in which neuronal GHS-R1a expression occurs. Also, dopamine is central to the actions of a variety of drugs of abuse, in particular of cocaine. Despite cocaine was first used by South America tribes for endurance and appetite suppression, little was known about the underlying mechanisms. Recent results show that one atypical receptor of unknown physiological function, the sigma-1 receptor (σ_1R), is a key piece in the puzzle constituted by interrelationships between cocaine and food seeking behaviors. First, this review covers the role of σ_1R in cocaine addiction via regulating dopaminergic transmission mediated by GPCRs. The second part of the paper reviews recent data showing how sigma receptors regulates ghrelinergic signaling. Finally, the review presents the novel and relevant evidence linking cocaine suppression of appetite with both cocaine binding to σ_1R and regulation via σ_1R of the function of dopamine and ghrelin GPCRs.

Apart from acting via inhibition of dopamine transporters in neurons, cocaine is able to interact with sigma-1 and sigma-2 receptors. Not only the physiological role of the two receptors is unknown but they are structurally unrelated despite they share the same name (sigma). This review focuses on sigma-1 receptor (σ_1R) as a mediator of the anorexigenic effect of cocaine. The receptor whose function remains a mystery displays interesting features such as i) its capacity to bind cocaine at physiologically-relevant concentrations and ii) evidence on modulating G-protein-coupled receptors (GPCRs), which in turn mediate the effects of drugs of abuse and of orexigenic hormones. Already in 1991, evidence was provided on blockade of stimulant effect of cocaine by targeting σ_1R (Menkel *et al.* 1991). It should be noted that treatment with synthetic drugs acting on σ_2 receptors decrease some of cocaine-induced effects (Matsumoto *et al.* 2007) and that antagonist of the receptors counteract induced locomotor stimulation in cocaine-administered mice (Lever *et al.* 2014). However, the research on σ_2 receptor research is still too preliminary to allow establishing solid link between the receptor and addiction mechanisms. One of the novel aspects in the review, which is based on recent results, is the advance in understanding how cocaine consumption reduces food seeking behavior. Apparently, this is due to direct interactions with the receptor for the hunger hormone, ghrelin.

The most striking and accepted effect of cocaine in brain is an increase in inter- and extra-synaptic dopamine concentration that leads to marked activation of dopamine receptors. These and many other neuronal receptors belonging to the family of G-protein-coupled receptors use cAMP as second messenger. IUPHAR information for sigma receptors shows their atypical nature as they do not use either cAMP or any other

second messenger as calcium ions or inositol-3-phosphate. As σ_1R are not coupled to any known signal transduction machinery IUPHAR states that "*there is only a modest pharmacological overlap and no structural convergence with the GPCRs*" (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=785). On the other hand, structurally different compounds may bind to σ_1R receptors at topologically intracellular sites. In fact, the binding of a drug seemingly acting on opioid receptors suggested that σ_1R receptors belonged to the opioid receptor family. The uniqueness of the receptor was first confirmed by lack of effect of opioid receptor antagonists and, secondly, by cloning and discovery of the non-GPCR structure. Solving of 3D structure (see details below) has confirmed some of the assumptions about these receptors and has provided brand new information related to atypical membrane insertion and seemingly physiological trimeric structure.

Evidence of relevant role of σ_1R receptors on cocaine addiction

A synthetic opioid-like molecule, SKF-10,047, displays psychomimetic activity while it does not selectively bind to μ- or k-opioid receptor. Although it was firstly assumed that the compound could bind to a novel opioid receptor (Martin *et al.* 1976), its effect was not blocked by nonselective opioid receptor antagonists (Vaupel 1983). The compound binds to a protein (σ_1R) whose cloning showed that it does not have seven transmembrane domains (Hanner *et al.* 1996). While waiting for more precise detailed of its physiological role, the σ_1R is considered a pluripotent modulator able to interact with different specific proteins (e.g. binding immunoglobulin protein -BiP-), cell components and/or signal transduction machineries (Su *et al.* 2016). σ_1R may interact with receptors for a variety of hormones/neurotransmitters and modulate either/or cell surface expression and function. Research on this protein is gaining momentum due to its potential as target to combat neuropathic pain (Mei & Pasternak 2002; Corbera *et al.* 2006; Sun *et al.* 2016). Agonistic activity in the case of σ_1R may be indirectly measured by subcellular translocation, establishment of protein-protein interactions and by regulation of ion channel activity (Wu & Bowen 2008; Kim *et al.* 2010; Navarro *et al.* 2010; Su *et al.* 2010). Indirect evidence also shows the involvement of σ_1R in disorders such as depression (Su *et al.* 2010). Also interesting is the correlation between a mutation in the receptor and debut and progression of juvenile lateral amyotrophic sclerosis (Al-Saif *et al.* 2011; Mavlyutov *et al.* 2013). More recently, a biophysical approach assessing homomerization of σ_1R and heteromerization of σ_1R with BiP has allowed distinguishing molecules with differential potential on stabilizing multimerization of the receptor and/or facilitating the σ_1R/BiP interaction (Yano *et al.* 2018). Despite the ambiguity of the words agonist/antagonist in the case of this specific receptor, the authors show that haloperidol and (+)-pentazocine have opposite effects, thus paving the way to effectively test or select molecules with "agonistic" versus opposite ("antagonistic") action.

Solving the 3D structure (Schmidt *et al.* 2016) provided another unexpected finding as

the σ_1R does not have, as previously hypothesized, two transmembrane domains and extracellular C- and N-terminal domains, but an extracellular N-terminal end a transmembrane α–helical domain and a C-terminal tail having a cupin-like β-barrel with a buried ligand-binding site. Crystals also show an arrangement consisting of three closely interacting σ_1R protomers. Despite homology (relatively poor) with a fungal sterol isomerase (Cobos *et al.* 2008), no catalytic activity has been yet allocated to σ_1R.

Due to the reports showing that the σ_1R may interact with receptors for a variety of hormones/neurotransmitters (see below), it was tempting to speculate that the role of the receptor may be regulation of the expression and function of cell surface receptors. (Kruse 2016) has recently reviewed the intriguing features of this protein, with no structural resemblance with any other membrane receptor, occluded ligand binding site, and resemblance to a yeast enzyme, yeast sterol isomerase. Remarkably, while the physiological function remains elusive and the endogenous ligand is yet to be discovered, cocaine binds to σ_1R, which mediate some effects of the drug (Skuza 1999; Shull 2002; Lever *et al.* 2016). The design of drugs impeding the interaction of cocaine with σ_1R is proposed to reduce drug-seeking behavior (Matsumoto *et al.* 2001a). Remarkably, σ_1R-mediated cocaine actions in the central nervous system are dependent on molecular interactions with dopamine and other neuron-expressed GPCRs.

Cocaine drives many of its effects via interacting with σ_1R

To our knowledge there are few reports addressing cocaine binding to σ_1R. It is however relevant that compared with other drugs of abuse the cocaine interaction with σ_1R was suspected several years ago (see (Hayashi & Su 2007) *for review*). Pioneering studies of cocaine binding to σ_1R were performed in 2001 ((Matsumoto *et al.* 2001b). Competition assays in radioligand binding studies showed that K_i of cocaine was 2.3 in mouse and 2.9 μM in rat brain. Values were similar in mice brain membranes (Lever *et al.* 2016) thus confirming that at "physiological" concentrations occurring after cocaine exposure the drug binds to σ_1R. Based on their results Matsumoto et al., 2003 proposed that σ_1R were targets to combat cocaine addiction. More recently, in a detailed study (Lever *et al.* 2016) showed that cocaine administration to mice reduces the binding to σ_1R of a radiolabeled ligand and it does so in a similar proportion in the 20 different brain regions analyzed by quantitative autoradiography. Average reduction of specific binding after i.p. administration of 100 μmol/kg cocaine was 54%. It should be noted that the σ_1R was expressed in all brain regions in a narrow range of 2-4 fmol/mg tissue.

A significant advance in addiction research has been provided by mechanisms of σ_1R linkage to the MAP kinase pathway. The receptor itself or upon cocaine binding cannot convey signals that impact on such key signaling pathway. The answer for this conundrum is the role of GPCRs that are able to directly binding to σ_1R. Then the most reasonable assumption is that cocaine binding to σ_1R produce conformational changes to cell surface GPCRs that are in direct contact with σ_1R. This possibility not only

affects signaling by also the overall biology of GPCRs relevant in addiction and also relevant in the control of food intake.

A first and in deep review on the role of σ_1R in the many different sides of cocaine addiction was provided in 2002 by (Romieu *et al.* 2002). The already existing data made authors state: "*σ_1R is not only necessary for acquisition of the cocaine-induced conditioned place preference, but that it is also implicated in its expression, confirming that activation of the σ_1R is induced during cocaine's early effects".* A very recent review summarizes the main properties of σ_1R and makes emphasis in its role as chaperone (see (Katz *et al.* 2017) and references therein). The review also presented the evidence of receptor involvement in three specific aspects of drug addiction, namely self-administration, discrimination and place-conditioning. We will here focus on the molecular mechanisms that are triggered by cocaine binding to σ_1R, but executed by other proteins. (Katz *et al.* 2017) propose that there is a "c*oncomitant targeting of both dopaminergic pathways and σR proteins".* However, recent evidence is more consistent with a more direct effect of cocaine on dopaminergic transmission and on other signaling systems operating in neurons of reward circuits. It is quite noteworthy that (Romieu *et al.* 2002) were able to intuitively notice this possibility as they also stated in their review: "*The σ_1R is activated consequently to dopamine reuptake blockade and is not sufficient to induce conditioned place preference (CPP) by itself. The mechanism of the* σ_1R *involvement in CPP and the selectivity toward the CPP-inducing drug remains however to be determined".* In fact, data in the last decade suggest new possibilities that have increased the knowledge of the molecular mechanisms underlying cocaine action. In what concerns dopaminergic signaling it may happen that cocaine binding to σ_1R produces an almost direct effect on dopaminergic transmission. This is possible because σ_1R directly interact with dopamine receptors and, accordingly, cocaine is binding to and affecting the structure and function of σ_1/dopamine receptor complexes.

Lessons from drugs behaving as σ_1R ligands

Pioneering studies related to the role of σ_1R in cocaine actions, showed that NPC 16377, a compound able to bind to these receptors was protective against toxic and behavioral effects of cocaine. NPC 16377 did not show any noticeable side effect while its efficacy was quite marked; for instance, it totally prevented the development of cocaine sensitization and significantly reduced diazepam-sensitive cocaine convulsions. Its effects were quite selective as it did not behave like NMDA receptor ligands and was not efficacious against the discriminative stimulus triggered by other drugs (Witkin *et al.* 1993). Similar results were obtained using different chemical structures, i.e. compounds able to reduce cocaine-induce hyperlocomotion and convulsions, were able to bind to σ_1R but not to receptors for neurotransmitters (McCracken *et al.* 1999; Shull 2002).

(Skuza 1999) tested different σ_1R ligands, *inter alia* rimcazole and panamesine, on cocaine-induced locomotor activity and convulsions. Whereas panamesine was effective

in the two actions of the drug, rimcazole increased the total time of cocaine-evoked convulsions and locomotor activity. Simultaneous administration of the assayed compounds showed that the effect of a given molecule could be reverted by another one. Skuza concluded: "*σ_2R subtype is involved in psychomotor stimulant effects of cocaine while σ_1R subtype participates in the cocaine-induced convulsions*". However, (Matsumoto et al., 2001) showed that rimcazole is acting via σ_1R. Authors tested different concentrations of analogs of rimcazole for their ability to bind to the two main targets of cocaine, dopamine transporters and σ_1R. Authors were successful in showing that drugs protective against convulsion-producing concentrations of cocaine were not acting via dopamine transporter inhibition but via binding to σ_1R. Also (Romieu *et al.* 2000) showed that two novel σ_1R ligands, BD1063 and BD1008, significantly increased the ED_{50} for the locomotor effects of cocaine. In a follow up study six analogues of BD1008 were tested against a variety of targets showing significant affinity for σ_1R, moderate for σ_2R and low or very low affinity for dopamine, opioid, NMDA and 5-hydroxytryptamine receptors or dopamine transporters (Matsumoto *et al.* 2004). The role of σ_1R was reinforced using an antisense oligodeoxynucleotide approach, which was efficacious in reducing the behavioral effects of cocaine (Matsumoto et al., 2001)**.** Taking into account all the cumulative evidence, antagonists of σ_1R were proposed as therapeutic agents against cocaine addiction (Matsumoto et al., 2003; Matsumoto et al., 2001; Maurice and Romieu, 2004). It should be however noted that σ_1R-based therapies may not work on acute symptoms and may be better suited to address drug sensitization. Indeed, a common phenomenon displayed by all ligands tested is reduction in psychostimulant-induced sensitization, not only to cocaine but to methamphetamine (Ujike *et al.* 1996).

σ_1R impact on signaling pathways originating at GPCRs and also on the function of ion channels; overall these functional interactions shape the behavioral and neuroanatomical plastic actions of cocaine (Kourrich *et al.* 2013). One of the first relevant aspects to consider is how σ_1R may affect dopaminergic signaling. As an example, the D_1 receptor mediated signaling and dopamine-induced inositol 1,4,5-trisphosphate production has been studied in dissociated neurons of the *nucleus accumbens* (NAc). The main finding was that the calcium mobilization produced by injection of inositol 1,4,5-trisphosphate was enhanced by cocaine in a σ_1R-dependent fashion. Not only cocaine increases the effects of agonists on cAMP levels but it alters the kinetics of the MAP kinase pathway engagement. Remarkably D_1 and σ_1 receptors do interact and the cocaine effects were dependent on σ_1R, a fact that was confirmed by using σ_1R KO mice (Navarro *et al.* 2010).

Cocaine, dopamine, dopamine receptors and dopamine heteroreceptor complexes

Dopamine is one of the main neurotransmitters in the brain, and their actions, mediated by dopamine receptors, are relevant for *inter alia* motor control and reward circuits targeted by addicting drugs. Pioneering electrophysiological studies by (Uchimura &

North 1990) showed that intracellular recordings in neurons triggered by 5-hydroxytryptamine or by dopamine in the NAc were affected by cocaine. The drug at doses in the 1-30 μM range affect dopamine D_1-receptor mediated hyperpolarization and D_2-receptor mediated depolarization. Cocaine was more effective, i.e. less doses were required, to alter the actions of 5-hydroxytryptamine.

In many circumstances a GPCR-containing heteroreceptor complex arises as the actual functional unit. In cocaine addiction, the engagement of σ_1R by cocaine and the interaction of these receptors with GPCRs has led to investigate how heteromers may contribute to the behavior and motor effects of the drug. As an example, complexes formed by dopamine D_1 and histamine H_3 receptors display particular properties as the heteromer is needed for signaling to the MAP kinase pathway (Ferrada *et al.* 2009). Cocaine alters heteromer function and it does so by a σ_1R-dependent mechanism (Moreno *et al.* 2014). These results probably reflect a macromolecular complex constituted by H_3, D_1 and σ_1 receptors whose structure and function becomes altered in the presence of cocaine. This interpretation is supported by the finding that antagonists of σ_1R restores the homeostatic interplay between H_3 and D_1 receptors and, therefore, supports therapeutic possibilities for σ_1R antagonists to combat drug addiction.

Cocaine affects signaling of other dopamine-receptor containing heteromers also in a σ_1R-dependent fashion. Adenosine A_{2A} and dopamine D_2 receptor heteromers were among the first identified GPCR heteromers (Hillion *et al.* 2002); they play a relevant role in the striatum and are the target for therapeutic approaches addressed to combat Parkinson's disease. On the one hand, energy transfer studies showed that cocaine altered the structure of adenosine receptor homomers and of adenosine/dopamine receptor heteromers. On the other hand the drug affected some but not all of the signaling pathways engaged by activation of D_2 receptors (Marcellino *et al.* 2010). Taking into account available data on the molecular mechanisms of cocaine actions (Borroto-Escuela *et al.* 2016) hypothesize that the drug is significantly altering the allosteric interactions occurring in homo- and heteroreceptor complexes, especially in those containing dopamine receptors. Taking also into account receptor distribution in different brain regions and changes in receptor expression after cocaine exposure the authors suggest that anti-cocaine actions of A_{2A} agonists do not depend on heteromerization with D_2 receptors, but that cocaine self-administration courses with a loss in the brake on the D_2 receptor signaling within the A_{2A}R-D_2R receptor heteroreceptor complex in dorsal striatopallidal GABA neurons. Apart from the well-known fact that cocaine markedly enhances the extracellular levels of dopamine in several regions of the central nervous system, little is known on the mechanism underlying drug addiction. Cocaine administration to non-human primates results in brain concentration peaks appearing as soon as 5 min to go back to basal at 30 min (Bradberry *et al.* 2000). A key region for addiction behavior establishment is the NAc.

A novel technique of heteromer detection shows that cocaine self-administration increases the expression of A_{2A}/D_2 and D_2/σ_1R complexes in the shell of the NAc whereas decrease those constituted by D_2 and σ_1R in the dorsal striatum (Borroto-

Escuela *et al.* 2017). Thus, self-administration likely increases in a regional selective way the expression of receptors that may establish direct interactions and have particular signaling pathways. It should be noted that σ_1R may form heteromers with D_2 receptors but not with other D_2-like receptors such as D_3 or D_4 receptors. The drug inhibits dopaminergic signaling in the striatum of wild type mice but not in the striatum of σ_1R KO mice. As commented below cocaine also affects D_1-receptor mediated signaling thus altering the D_1-D_2 functional balance required for proper motor control (Navarro *et al.* 2013). This σ_1R mediated effect on striatal dopaminergic signaling is probably one of the main factors underlying locomotor actions of cocaine.

A small but significant percentage of neurons in the NAc express D_1 and D_2 dopamine receptors. There is dispute on the possibility that D_1 and D_2 may form heteroreceptor complexes in the motor control brain areas (Lee *et al.* 2004; Rashid *et al.* 2007; Perreault *et al.* 2010; George *et al.* 2014; Frederick *et al.* 2015; Rico *et al.* 2016). The most likely hypothesis is that 10-20% of neurons in the NAc express both receptors that establish heteroreceptor complexes shifting dopaminergic transmission from cAMP- to Ca^{2+}-dependent signaling (Hasbi *et al.* 2009). As the increase of dopamine in this nucleus is fundamental for addiction and a significant amount of these cells express the two dopamine receptors, it is hypothesized that D_1/D_2 heteroreceptor complexes are important for establishment of cocaine seeking behavior. In line with this hypothesis, recent studies show that disruption of the heteromer has profound consequences in animals treated with cocaine. Intracerebroventricular administration of disrupting peptides, induces, sustains, accelerates and exacerbates the incentive motivational and locomotor activating effects of cocaine in a self-administration paradigm. The blocking peptides were also able to increase ΔFosB expression in the NAc. These findings suggest a model for tonic inhibition of basal and cocaine-induced reward (Perreault *et al.* 2016). Future experiments should address the question of whether σ_1R may interact with D_1/D_2 heteroreceptors and mediate cocaine actions in neurons expressing the two dopamine receptors.

Cocaine alters mitogen activated protein kinase (MAP) pathway via GPCRs

One of the common factors in drug addiction is the involvement of the MAP kinase pathway. Many drugs of abuse and, also, natural compounds with psychoactive effects impact on the pathway. Structurally different drugs such as tetrahydrocannabinol (THC) or cocaine increase the phosphorylation of extracellular signal-regulated kinases (ERKs) in different brain regions, and pharmacological blockade of the pathway impairs conditioned place preference. Furthermore, activation of the MAP kinase pathway seems to contribute to the cerebral plastic changes induced by drugs of abuse (see (Valjent *et al.* 2004) and references therein). Activation of the MAP kinase pathway is needed for establishing an association between drug consumption and conditioned place preference (Valjent *et al.* 2006; Du *et al.* 2017). Of the two predominant ERK isoforms, ERK2 seems more directly involved in plasticity changes produced by repeated exposure to cocaine. This conclusion arises from data in ERK1 KO mice, which display

a facilitation of acquisition of cocaine conditioned place preference and of locomotor sensitization (Ferguson *et al.* 2006). Also interesting is the finding that dopamine D_1 receptor mediates cocaine-induced long-term plasticity in the NAc (Zhang *et al.* 2017). On the one hand, dopamine supersensitivity subsequent to cocaine consumption alters the homeostasis of the pathway. Although the temporal course of cocaine-induced increase of dopamine occurs mainly in ventral striatum (Kalivas 1993; Kalivas & Duffy 1993)**,** it affects other brain regions in virtue of the volume transmission mechanism defined by (Agnati *et al.* 1986); dopamine does so probably in close but diverse regions within the reward circuits. On the other hand, whereas THC or caffeine acts by direct binding to GPCRs, a direct effect of cocaine of those receptors is unlikely. Interestingly enough, cocaine binding to σ_1R do impact on the signal transduction mechanisms that originate at GPCRs and regulate the MAP kinase and mTOR pathways (see (Franco *et al.* 2017) and references therein). In the amygdala, which is also is affected in cocaine addiction, acute and chronic drug administration produced different patterns of immediate early gene expression by mechanisms dependent on ERK activation (Radwanska *et al.* 2005). Therefore, the MAP kinase pathway arises as a key mediator of the central actions produced in response to cocaine administration.

Orexin and ghrelin receptors

A review of the literature on orexin/hypocretin physiology and orexin receptor pharmacology made (Kukkonen and Leonard, 2014; Leonard and Kukkonen, 2014) suggest that orexigenic receptors could be therapeutic targets of food disorders and drug addiction. Such hypothesis is partly based on the fact that orexin G-protein-coupled receptor pharmacology is multifaceted ranging from activation of canonical signaling pathways to regulation of ion fluxes.

Cocaine-seeking behavior involves several mediators such as orexin-A and corticotropin-releasing factor (CRF) acting in the ventral tegmental area. Again, receptors related with cocaine effects have the possibility of forming heteromers whose function is affected by the drug. As a relevant example CRF1 (CRF1R) interact with orexin OX1 receptors and the interaction results in a negative crosstalk between orexin-A and CRF; these neuroregulators/hormones regulate dendritic dopamine release in the ventral tegmental area. But the σ_1R also interacts with GPCR heteroreceptor complexes and cocaine binding to σ_1R sensitizes cells to excitatory effects of CRF and orexin A; the mechanisms consists of the cross-talk between CRF1-OX1 receptors (Navarro *et al.* 2015). These results reflect an interplay between addiction, stress and, importantly, control of food intake by orexigenic factors.

Ghrelin is a peptide hormone that controls food intake and energy homeostasis (Figure 1). Its action is mediated by specific receptors that have received a variety of denominations, *inter alia* growth hormone-releasing peptide or growth hormone secretagogue receptor. Ghrelin receptors belong to the superfamily of G-protein-coupled receptors (GPCRs) and, up to date, only one type has been identified. The full length

388 amino-acid-long human ghrelin receptor containing seven transmembrane domains is known as GHS-R1a, to differentiate it from the GHS-R1b splice variant, which is 289 amino acid long and lacks the 5th and 6th transmembrane (TM) domains. These TM domains are required for coupling to heterotrimeric G proteins and, therefore, ghrelin cannot signal via GHS-R1b receptors (Mary *et al.* 2013). The truncated variant seems to serve as modulator of GHS-R1a surface expression and signaling. In fact, GHS-R1b is expressed in the same cells than GHS-R1a and both receptors interact to form heteromer receptor signaling units (Mary *et al.* 2013). It has been reported that GHS-R1b negatively influences ghrelin action by reducing surface expression of functional G-protein-coupled ghrelin receptors (Chow *et al.* 2012) and by allosteric interactions that reduce the efficacy of the hormone (Mary *et al.* 2013).

In a detailed review (Schellekens *et al.* 2013a) nicely summarize how activation of specific receptors in the brain shapes the many actions of the so-called hunger hormone. The link between ghrelin and dopaminergic transmission in reward circuits is highlighted: "*The ghrelin signaling system has recently been suggested to play a key role at the interface of homeostatic control of appetite and the hedonic aspects of food intake, as a critical role for ghrelin in dopaminergic mesolimbic circuits involved in reward signaling has emerged*". They also point out that ghrelin receptors may establish interactions with other proteins that may shape the central effects of ghrelin (Schellekens *et al.* 2013a, b)**.** In fact, ghrelin receptors may establish direct protein-protein interactions with a variety of GPCRs: dopamine, melanocortin, prostanoid, serotonin, somatostatin and neurotensin receptors ((Borroto-Escuela *et al.* 2014); see www.gpcr-hetnet.com and references therein). In a detailed study in which complexes formed by GHS-R1a-GHS-R1b and dopamine D_1 receptors were detected, differences in the function of ghrelin receptor signaling was found in hippocampal versus striatal neurons. In the latter D_1 receptors were involved in GHS-R1a-Gs/olf coupling. Thus, the dopamine receptor may switch from $G_{i/o}$ to $G_{s/olf}$ coupling but only if GHS-R1b is also expressed (Navarro *et al.* 2016). It then appears that anything affecting D_1 receptor-mediated signaling may in turn affect ghrelin actions.

Links between drug addiction and anorexic behavior

Used today as recreational drug, cocaine was first consumed by humans in the form of *Coca* leaves. Indeed, indigenous peoples of South American knew that chewing coca leaves was key for keeping their life style, especially in living within high mountains. Coca served to cope with the harsh living conditions, for instance when people had to travel long distances and cross Andean mountains with reduced weight and little food. Despite such ancient knowledge, i.e. the appetite suppressant action of cocaine consumption, the molecular basis of hunger dissipation by cocaine have remained elusive. Interestingly, years ago (Cottone *et al.* 2012) showed that antagonists of σ_1R blocked compulsive eating behavior in rats. These early results fit nicely with those of a recent report that has provided insight into the underlying molecular mechanisms.

It has been established that addiction and eating disorders (e.g. binge eating, anorexia, bulimia) share a central control that involves reward circuits in the brain. This leads to bidirectional influences: on one hand, previous history of binge eating predisposes to the addictive behavior whereas the cessation of exposure to drugs of abuse leads to persistent proclivity towards reward-providing activities, including the intake of palatable foods. From ancient times, it has been known that the use of cocaine is associated with decreased food intake, with the inversion after the drug use is stopped. This creates a vicious circle in which the weight gain that follows the discontinuance of cocaine use secondarily causes a significant distress which can make a patient more prone to the relapse. Many uncertainties remain about the biological substrate of these changes, particularly at the level of signaling systems involved. Thus, establishing the molecular mechanisms of such complex interactions is of immense biological and medical importance.

Exciting new findings substantiate the concept that the receptors for "hunger" hormone, ghrelin, on neurons in the CNS directly interact with σ_1R. The results provide solid evidence of the anorexic effect of cocaine being mediated by ghrelin receptors, that arise as both key players in the central control of food/energy intake and sensitive to σ_1R-mediated cocaine effects. Importantly, the ghrelin/σ_1R interaction creates qualitatively new, higher-order structures, with altered signaling properties. Unraveling the mechanisms applicable in this setting may ultimately translate into the new approaches relevant for numerous other areas of research (e.g. endocrinology, behavioral neuroscience), as well as for addressing the societal impact of these disorders, particularly in the youth population.

Cocaine affects ghrelin receptor traffic and function via σ_1R

A first relevant piece of information concerning cocaine and ghrelin receptors is the fact that the drug alters the expression of GHS-R1a at the membrane level. Cocaine at a physiologically relevant dose (Navarro *et al.* 2010) is able increase plasma membrane expression of σ_1R. The so-called σ_1R agonists do the same effect thus confirming a very specific effect. Remarkably both cocaine and σ_1R agonists increase the colocalization of the two receptors at the cell surface. Accordingly, any drug interacting in an "agonistic" manner with σ_1R is able to concomitantly affect coexpression of the two receptors at the cell surface where they keep direct-direct interactions a deduced from assays in brain sections for cocaine-treated animals and in an heterologous expression system (Aguinaga *et al.* 2018); manuscript in revision; first version included as Supplementary information).

The *in situ* proximity ligation assay (Borroto-Escuela et al., 2016) allows determining the occurrence of receptor complexes in natural sources. The technique showed occurrence of σ_1/GHS-R1a receptor heteromeric complexes in rat brain sections. 11% of cells in rat striatum displayed the fluorescent signal corresponding to heteromers. In animals chronically treated with the addictive drug the percentage of positive cells

increased to 61% and the amount of signal, measured as red clusters also increased. The acute treatment lead to a more marked increase in both percentage of labelled cells (76%) and degree of labelling (3.2-fold increase). The results are consistent with both the occurrence of σ_1/ghrelin receptor complexes and a marked upregulation of those complexes upon acute or chronic cocaine treatment. Upregulation of σ_1/GHS-R1a receptor heteromeric complexes was also obtained in primary cultures of primary striatal neurons treated with cocaine. This finding led to the hypothesis that cocaine binding to ghrelin receptors could affect the ghrelin-receptor mediated signaling. The hypothesis addressed both in a heterologous expression system and primary cultures of striatal neurons led to similar findings.

Consistent with the coupling of ghrelin receptor to heterotrimeric Gi proteins, its activation using the endogenous ghrelin in the presence of forskolin significantly decreases intracellular cAMP levels. Signaling via Gi was blocked by both agonists of σ_1R and by cocaine. Signal transduction in neurons expressing GHS-R1a leads to activation of the MAP kinase pathway. This signaling, i.e. ERK phosphorylation triggered by GHS-R1a activation was not only inhibited by ghrelin receptor antagonists but by cocaine and σ_1R agonists (Figure 2). Therefore, both G-dependent and G-independent signaling becomes compromised by cocaine binding to σ_1R and disappear when σ_1R is silenced by a siRNA methodology.

A further relevant question was to know whether the cocaine effects were mediated by either σ_1 containing heteroreceptor complexes or by indirect mechanisms involving second messengers or other signaling molecules. Cocaine acting as agonist of the receptor may stabilize its trimeric structure (Gromek *et al.* 2014). Taking advantage of the (trimeric) 3D-structure of σ_1R (Schmidt *et al.* 2016) a model was proposed for the σ_1R- GHS-R1a interaction. The model predicted that the transmembrane 1 (TM1) domain of the ghrelin receptor form participates in the interaction interface whereas TM7 does not. The issue can be addresses taking advantage of interfering peptides. They have been successfully used to disrupt the structure of interactions involving GPCRs (Navarro *et al.* 2018). The peptides consist of receptor TM sequences followed by a short sequence of the cell-penetrating HIV transactivator of transcription (TAT) that is responsible of cell membrane penetrance (Schwarze *et al.* 1999). In agreement with the model provided for σ_1R- GHS-R1a heteromers, the TAT-TM1 but not the TAT-TM7 achieved a lack of effect of cocaine on GHS-R1a-mediated signaling while the ghrelin action was still blocked by the selective ghrelin receptor antagonist. In summary, at the mechanistic level the cocaine blockade of ghrelin action occurs at a proximal level in CNS neurons by a direct action of σ_1R directly interacting with ghrelin receptors (Figure 2).

Conflict of interests

Authors declare no conflict of interests.

Acknowledgements

This work was supported by grant from Spanish Ministry of Innovation and Competitiveness (MINECO) (Ref. No. BFU2015-64405-R; it may contain EU FEDER funds)

References

Agnati LF, Fuxe K, Zoli M, Ozini I, Toffano G & Ferraguti F 1986 A correlation analysis of the regional distribution of central enkephalin and beta-endorphin immunoreactive terminals and of opiate receptors in adult and old male rats. Evidence for the existence of two main types of communication in the central nervous. *Acta Physiologica Scandinavica* **128** 201–207. (doi:10.1111/j.1748-1716.1986.tb07967.x)

Aguinaga D, Medrano M, Cordomí A, Jiménez-Rosés M, Angelats E, Casanovas M, Vega-Quiroga I, Canela EI, Petrovic M, Gysling K *et al.* 2018 Cocaine blocks effects of hunger hormone, ghrelin, via interaction with neuronal sigma-1 receptors. *Molecular Neurobiology* **Epub ahead**. (doi:10.1007/s12035-018-1140-7)

Al-Saif A, Al-Mohanna F & Bohlega S 2011 A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. *Annals of Neurology* **70** 913–919. (doi:10.1002/ana.22534)

Borroto-escuela DO, Hagman B, Woolfenden M, Pinton L, Jiménez-beristain A, Ofl J, Narvaez M, Palma M Di, Feltmann K, Sartini S *et al.* 2016 Receptor and Ion Channel Detection in the Brain. *Neuromethods* **110** 109–124. (doi:10.1007/978-1-4939-3064-7)

Borroto-Escuela DO, Brito I, Romero-Fernandez W, Di Palma M, Oflijan J, Skieterska K, Duchou J, Van Craenenbroeck K, Suárez-Boomgaard D, Rivera A *et al.* 2014 The G protein-coupled receptor heterodimer network (GPCR-HetNet) and its hub components. *International Journal of Molecular Sciences* **15** 8570–8590. (doi:10.3390/ijms15058570)

Borroto-Escuela DO, Wydra K, Pintsuk J, Narvaez M, Corrales F, Zaniewska M, Agnati LF, Franco R, Tanganelli S, Ferraro L *et al.* 2016 Understanding the Functional Plasticity in Neural Networks of the Basal Ganglia in Cocaine Use Disorder: A Role for Allosteric Receptor-Receptor Interactions in A2A-D2 Heteroreceptor Complexes. *Neural Plasticity* **2016** 1–12. (doi:10.1155/2016/4827268)

Borroto-Escuela DO, Narváez M, Wydra K, Pintsuk J, Pinton L, Jimenez-Beristain A, Di Palma M, Jastrzębska J, Filip M & Fuxe K 2017 Cocaine self-administration specifically increases A2AR-D2R and D2R-sigma1R heteroreceptor complexes in the rat nucleus accumbens shell. Relevance for cocaine use disorder. *Pharmacology Biochemistry and Behavior* **155** 24–31. (doi:10.1016/j.pbb.2017.03.003)

Bradberry CW, Barrett-Larimore RL, Jatlow P & Rubino SR 2000 Impact of self-administered cocaine and cocaine cues on extracellular dopamine in mesolimbic and sensorimotor striatum in rhesus monkeys. *Journal of Neuroscience* **20** 3874–3883. (doi:20/10/3874 [pii])

Chow KBS, Sun J, Chu KM, Tai Cheung W, Cheng CHK & Wise H 2012 The truncated ghrelin receptor polypeptide (GHS-R1b) is localized in the endoplasmic reticulum where it forms heterodimers with ghrelin receptors (GHS-R1a) to attenuate their cell surface expression. *Molecular and Cellular Endocrinology* **348** 247–254. (doi:10.1016/j.mce.2011.08.034)

Cobos E, Entrena J, Nieto F, Cendan C & Pozo E 2008 Pharmacology and Therapeutic Potential of Sigma1 Receptor Ligands. *Current Neuropharmacology* **6** 344–366. (doi:10.2174/157015908787386113)

Corbera J, Vaño D, Martínez D, Vela JM, Zamanillo D, Dordal A, Andreu F, Hernandez E, Perez R, Escriche M *et al.* 2006 A medicinal-chemistry-guided approach to selective and druglike sigma 1 ligands. *ChemMedChem* **1** 140–154. (doi:10.1002/cmdc.200500034)

Cottone P, Wang X, Park JW, Valenza M, Blasio A, Kwak J, Iyer MR, Steardo L, Rice KC, Hayashi T *et al.* 2012 Antagonism of sigma-1 receptors blocks compulsive-like eating. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* **37** 2593–2604. (doi:10.1038/npp.2012.89)

Du Y, Du L, Cao J, Hölscher C, Feng Y, Su H, Wang Y & Yun KM 2017 Levo-tetrahydropalmatine inhibits the acquisition of ketamine-induced conditioned place preference by regulating the expression of ERK and CREB phosphorylation in rats. *Behavioural Brain Research* **317** 367–373. (doi:10.1016/j.bbr.2016.10.001)

Ferguson SM, Fasano S, Yang P, Brambilla R & Robinson TE 2006 Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. *Neuropsychopharmacology* **31** 2660–2668. (doi:10.1038/sj.npp.1301014)

Ferrada C, Moreno E, Casadó V, Bongers G, Cortés A, Mallol J, Canela EI, Leurs R, Ferré S, Lluís C *et al.* 2009 Marked changes in signal transduction upon heteromerization of dopamine D1 and histamine H3 receptors. *British Journal of Pharmacology* **157** 64–75. (doi:10.1111/j.1476-5381.2009.00152.x)

Franco R, Martínez-Pinilla E, Navarro G & Zamarbide M 2017 Potential of GPCRs to modulate MAPK and mTOR pathways in Alzheimer's disease. *Progress in Neurobiology* **149**–**150** 21–38. (doi:10.1016/j.pneurobio.2017.01.004)

Frederick AL, Yano H, Trifilieff P, Vishwasrao HD, Biezonski D, Mészáros J, Urizar E, Sibley DR, Kellendonk C, Sonntag KC *et al.* 2015 Evidence against dopamine D1/D2 receptor heteromers. *Molecular Psychiatry*. (doi:10.1038/mp.2014.166)

George SRSR, Kern A, Smith RGRG & Franco R 2014 Dopamine receptor heteromeric complexes and their emerging functions. *Progress in Brain Research* **211** 183–200. (doi:10.1016/B978-0-444-63425-2.00008-8)

Gromek KA, Suchy FP, Meddaugh HR, Wrobel RL, LaPointe LM, Chu UB, Primm JG, Ruoho AE, Senes A & Fox BG 2014 The oligomeric states of the purified sigma-1 receptor are stabilized by ligands. *The Journal of Biological Chemistry* **289** 20333–20344. (doi:10.1074/jbc.M113.537993)

Hanner M, Moebius FF, Flandorfer A, Knaus HG, Striessnig J, Kempner E & Glossmann H 1996 Purification, molecular cloning, and expression of the mammalian sigma1-binding site. *Proceedings of the National Academy of Sciences of the United States of America* **93** 8072–8077.

Hasbi A, Fan T, Alijaniaram M, Nguyen T, Perreault ML, O'Dowd BF & George SR 2009 Calcium signaling cascade links dopamine D1-D2 receptor heteromer to striatal BDNF production and neuronal growth. *Proceedings of the National Academy of Sciences of the United States of America* **106** 21377–21382. (doi:10.1073/pnas.0903676106)

Hayashi T & Su TP 2007 Sigma-1 Receptor Chaperones at the ER- Mitochondrion Interface Regulate Ca2+ Signaling and Cell Survival. *Cell* **131** 596–610. (doi:10.1016/j.cell.2007.08.036)

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A, Hansson A, Watson S, Olah ME, Mallol J *et al.* 2002 Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. *The Journal of Biological Chemistry* **277** 18091–18097. (doi:10.1074/jbc.M107731200)

Kalivas PW 1993 Time Course of Extracellular Dopamine to Cocaine . II . Dopamine Perikarya and Behavioral Sensitization. *Brain Research* **73** 276–284.

Kalivas PW & Duffy P 1993 Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* **13** 266–275.

Katz JL, Hiranita T, Hong WC, Job MO & McCurdy CR 2017 A role for sigma receptors in stimulant self-administration and addiction. *Handbook of Experimental Pharmacology* **244** 177–218. (doi:10.1007/164_2016_94)

Kim FJ, Kovalyshyn I, Burgman M, Neilan C, Chien C-C & Pasternak GW 2010 1 Receptor Modulation of G-Protein-Coupled Receptor Signaling: Potentiation of Opioid Transduction Independent from Receptor Binding. *Molecular Pharmacology* **77** 695–703. (doi:10.1124/mol.109.057083)

Kourrich S, Hayashi T, Chuang JY, Tsai SY, Su TP & Bonci A 2013 Dynamic interaction between sigma-1 receptor and Kv1.2 shapes neuronal and behavioral responses to cocaine. *Cell* **152** 236–247. (doi:10.1016/j.cell.2012.12.004)

Kruse A 2016 Structural Insights into Sigma1 Function. In *Handbook of Experimental Pharmacology*. (doi:10.1007/164_2016_95)

Kukkonen JP & Leonard CS 2014 Orexin/hypocretin receptor signalling cascades. *British Journal of Pharmacology* **171** 314–331. (doi:10.1111/bph.12324)

Lee SP, So CH, Rashid AJ, Varghese G, Cheng R, Lança AJ, O'Dowd BF & George SR 2004 Dopamine D1 and D2 receptor Co-activation generates a novel phospholipase C-mediated calcium signal. *The Journal of Biological Chemistry* **279** 35671–35678. (doi:10.1074/jbc.M401923200)

Leonard CS & Kukkonen JP 2014 Orexin/hypocretin receptor signalling: A functional perspective. *British Journal of Pharmacology* **171** 294–313. (doi:10.1111/bph.12296)

Lever JR, Miller DK, Green CL, Fergason-cantrell EA, Watkinson LD, Carmack TL,

633 Fan K hsien & Lever SZ 2014 A selective sigma-2 receptor ligand antagonizes
634 cocaine-induced hyperlocomotion in mice. *Synapse* **68** 73–84.
635 (doi:10.1002/syn.21717)

636 Lever JR, Fergason-Cantrell EA, Watkinson LD, Carmack TL, Lord SA, Xu R, Miller
637 DK & Lever SZ 2016 Cocaine occupancy of sigma 1 receptors and dopamine
638 transporters in mice. *Synapse* **70** 98–111. (doi:10.1002/syn.21877)

639 Marcellino D, Navarro G, Sahlholm K, Nilsson J, Agnati LF, Canela EI, Lluís C,
640 Århem P, Franco R & Fuxe K 2010 Cocaine produces D2R-mediated
641 conformational changes in the adenosine A(2A)R-dopamine D2R heteromer.
642 *Biochemical and Biophysical Research Communications* **394** 988–992.
643 (doi:10.1016/j.bbrc.2010.03.104)

644 Martin WR, Eades CG, Thompson JA, Huppler RE & Gilbert PE 1976 The effects of
645 morphine- and nalorphine- like drugs in the nondependent and morphine-
646 dependent chronic spinal dog. *The Journal of Pharmacology and Experimental*
647 *Therapeutics* **197** 517–532.

648 Mary S, Fehrentz J-A, Damian M, Gaibelet G, Orcel H, Verdié P, Mouillac B, Martinez
649 J, Marie J & Banères J-L 2013 Heterodimerization with Its splice variant blocks
650 the ghrelin receptor 1a in a non-signaling conformation: a study with a purified
651 heterodimer assembled into lipid discs. *The Journal of Biological Chemistry* **288**
652 24656–24665. (doi:10.1074/jbc.M113.453423)

653 Matsumoto RR, Hewett KL, Pouw B, Bowen WD, Husbands SM, Cao JJ & Newman
654 AH 2001a Rimcazole analogs attenuate the convulsive effects of cocaine:
655 correlation with binding to sigma receptors rather than dopamine transporters.
656 *Neuropharmacology* **41** 878–886.

657 Matsumoto RR, McCracken KA, Pouw B, Miller J, Bowen WD, Williams W & De
658 Costa BR 2001b N-alkyl substituted analogs of the σ receptor ligand BD1008 and
659 traditional σ receptor ligands affect cocaine-induced convulsions and lethality in
660 mice. *European Journal of Pharmacology* **411** 261–273. (doi:10.1016/S0014-
661 2999(00)00917-1)

662 Matsumoto RR, Hewett KL, Pouw B, Bowen WD, Husbands SM, Cao JJ & Newman
663 AH 2001c Rimcazole analogs attenuate the convulsive effects of cocaine:
664 Correlation with binding to sigma receptors rather than dopamine transporters.
665 *Neuropharmacology* **41** 878–886. (doi:10.1016/S0028-3908(01)00116-2)

666 Matsumoto RR, McCracken KA, Friedman MJ, Pouw B, De Costa BR & Bowen WD
667 2001d Conformationally restricted analogs of BD1008 and an antisense
668 oligodeoxynucleotide targeting σ1 receptors produce anti-cocaine effects in mice.
669 *European Journal of Pharmacology* **419** 163–174. (doi:10.1016/S0014-
670 2999(01)00968-2)

671 Matsumoto RR, Liu Y, Lerner M, Howard EW & Brackett DJ 2003 σ receptors:
672 Potential medications development target for anti-cocaine agents. *European*
673 *Journal of Pharmacology* **469** 1–12. (doi:10.1016/S0014-2999(03)01723-0)

674 Matsumoto RR, Gilmore DL, Pouw B, Bowen WD, Williams W, Kausar A & Coop A
675 2004 Novel analogs of the σ receptor ligand BD1008 attenuate cocaine-induced
676 toxicity in mice. *European Journal of Pharmacology* **492** 21–26.
677 (doi:10.1016/j.ejphar.2004.03.037)

Matsumoto RR, Pouw B, Mack AL, Daniels A & Coop A 2007 Effects of UMB24 and (+/-)-SM 21, putative sigma2-preferring antagonists, on behavioral toxic and stimulant effects of cocaine in mice. *Pharmacology, Biochemistry, and Behavior* **86** 86–91. (doi:10.1016/j.pbb.2006.12.011)

Maurice T & Romieu P 2004 Involvement of the sigma1 receptor in the appetitive effects of cocaine. *Pharmacopsychiatry* **37** 198–207. (doi:10.1055/s-2004-832678)

Mavlyutov TA, Epstein ML, Verbny YI, Huerta MS, Zaitoun I, Ziskind-Conhaim L & Ruoho AE 2013 Lack of sigma-1 receptor exacerbates ALS progression in mice. *Neuroscience* **240** 129–134. (doi:10.1016/j.neuroscience.2013.02.035)

McCracken KA, Bowen WD, De Costa BR & Matsumoto RR 1999 Two novel σ receptor ligands, BD1047 and LR172, attenuate cocaine-induced toxicity and locomotor activity. *European Journal of Pharmacology* **370** 225–232. (doi:10.1016/S0014-2999(99)00113-2)

Mei J & Pasternak GW 2002 Sigma1 receptor modulation of opioid analgesia in the mouse. *The Journal of Pharmacology and Experimental Therapeutics* **300** 1070–1074. (doi:10.1124/jpet.300.3.1070)

Menkel M, Terry P, Pontecorvo M, Katz JL & Witkin JM 1991 Selective σ ligands block stimulant effects of cocaine. *European Journal of Pharmacology* **201** 251–252. (doi:10.1016/0014-2999(91)90355-T)

Moreno E, Moreno-Delgado D, Navarro G, Hoffmann HM, Fuentes S, Rosell-Vilar S, Gasperini P, Rodríguez-Ruiz M, Medrano M, Mallol J *et al.* 2014 Cocaine Disrupts Histamine H3 Receptor Modulation of Dopamine D1 Receptor Signaling: σ1-D1-H3 Receptor Complexes as Key Targets for Reducing Cocaine's Effects. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* **34** 3545–3558. (doi:10.1523/JNEUROSCI.4147-13.2014)

Navarro G, Moreno E, Aymerich M, Marcellino D, McCormick PJ, Mallol J, Cortes A, Casado V, Canela EI, Ortiz J *et al.* 2010 Direct involvement of -1 receptors in the dopamine D1 receptor-mediated effects of cocaine. *Proceedings of the National Academy of Sciences* **107** 18676–18681. (doi:10.1073/pnas.1008911107)

Navarro G, Moreno E, Bonaventura J, Brugarolas M, Farré D, Aguinaga D, Mallol J, Cortés A, Casadó V, Lluís C *et al.* 2013 Cocaine Inhibits Dopamine D2 Receptor Signaling via Sigma-1-D2 Receptor Heteromers. *PloS One* **8** e61245. (doi:10.1371/journal.pone.0061245)

Navarro G, Quiroz C, Moreno-Delgado D, Sierakowiak A, McDowell K, Moreno E, Rea W, Cai N-S, Aguinaga D, Howell LA *et al.* 2015 Orexin-corticotropin-releasing factor receptor heteromers in the ventral tegmental area as targets for cocaine. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* **35** 6639–6653. (doi:10.1523/JNEUROSCI.4364-14.2015)

Navarro G, Aguinaga D, Angelats E, Medrano M, Moreno E, Mallol J, Cortés A, Canela EI, Casadó V, McCormick PJ *et al.* 2016 A significant role of the truncated ghrelin receptor GHS-R1b in ghrelin-induced signaling in neurons. *Journal of Biological Chemistry* **291** 13048–13062. (doi:10.1074/jbc.M116.715144)

Navarro G, Cordomí A, Brugarolas M, Moreno E, Aguinaga D, Pérez-Benito L, Ferre S, Cortés A, Casadó V, Mallol J *et al.* 2018 Cross-communication between Gi and Gs in a G-protein-coupled receptor heterotetramer guided by a receptor C-terminal

723 domain. *BMC Biology* **16:24** 1–15. (doi:10.1186/s12915-018-0491-x)

724 Perreault ML, Hasbi A, Alijaniaram M, Fan T, Varghese G, Fletcher PJ, Seeman P,
725 O'Dowd BF & George SR 2010 The dopamine D1-D2 receptor heteromer
726 localizes in dynorphin/enkephalin neurons: increased high affinity state following
727 amphetamine and in schizophrenia. *The Journal of Biological Chemistry* **285**
728 36625–36634. (doi:10.1074/jbc.M110.159954)

729 Perreault ML, Hasbi A, Shen MYFFMYF, Fan T, Navarro G, Fletcher PJPJ, Franco R,
730 Lanciego JLJL & George SRSR 2016 Disruption of a dopamine receptor complex
731 amplifies the actions of cocaine. *European Neuropsychopharmacology* **26** 1366–
732 1377. (doi:10.1016/j.euroneuro.2016.07.008)

733 Radwanska K, Caboche J & Kaczmarek L 2005 Extracellular signal-regulated kinases
734 (ERKs) modulate cocaine-induced gene expression in the mouse amygdala.
735 *European Journal of Neuroscience* **22** 939–948. (doi:10.1111/j.1460-
736 9568.2005.04286.x)

737 Rashid AJ, So CH, Kong MMC, Furtak T, El-Ghundi M, Cheng R, O'Dowd BF &
738 George SR 2007 D1-D2 dopamine receptor heterooligomers with unique
739 pharmacology are coupled to rapid activation of Gq/11 in the striatum.
740 *Proceedings of the National Academy of Sciences of the United States of America*
741 **104** 654–659. (doi:10.1073/pnas.0604049104)

742 Rico AJ, Dopeso-Reyes IG, Martínez-Pinilla E, Sucunza D, Pignataro D, Roda E,
743 Marín-Ramos D, Labandeira-García JL, George SR, Franco R *et al.* 2016
744 Neurochemical evidence supporting dopamine D1–D2 receptor heteromers in the
745 striatum of the long-tailed macaque: changes following dopaminergic
746 manipulation. *Brain Structure and Function* **222** 1–18. (doi:10.1007/s00429-016-
747 1306-x)

748 Romieu P, Martin-Fardon R & Maurice T 2000 Involvement of the sigma1 receptor in
749 the cocaine-induced conditioned place preference. *Neuroreport* **11** 2885–2888.
750 (doi:10.1097/00001756-200009110-00011)

751 Romieu P, Phan VL, Martin-Fardon R & Maurice T 2002 Involvement of the
752 sigma1receptor in cocaine-induced conditioned place preference: Possible
753 dependence on dopamine uptake blockade. *Neuropsychopharmacology* **26** 444–
754 455. (doi:10.1016/S0893-133X(01)00391-8)

755 Schellekens H, Dinan TG & Cryan JF 2013a Taking two to tango: a role for ghrelin
756 receptor heterodimerization in stress and reward. *Frontiers in Neuroscience* **7** 148.
757 (doi:10.3389/fnins.2013.00148)

758 Schellekens H, van Oeffelen WEPA, Dinan TG & Cryan JF 2013b Promiscuous
759 dimerization of the growth hormone secretagogue receptor (GHS-R1a) attenuates
760 ghrelin-mediated signaling. *The Journal of Biological Chemistry* **288** 181–191.
761 (doi:10.1074/jbc.M112.382473)

762 Schmidt HR, Zheng S, Gurpinar E, Koehl A, Manglik A & Kruse AC 2016 Crystal
763 structure of the human σ1 receptor. *Nature* **532** 527–530.
764 (doi:10.1038/nature17391)

765 Schwarze SR, Ho A, Vocero-Akbani A & Dowdy SF 1999 In vivo protein transduction:
766 Delivery of a biologically active protein into the mouse. *Science* **285** 1569–1572.
767 (doi:10.1126/science.285.5433.1569)

768 Shull KR 2002 Contact mechanics and the adhesion of soft solids. *Materials Science*
769 *and Engineering: R: Reports* **36** 1–45. (doi:10.1016/S0927-796X(01)00039-0)

770 Skuza G 1999 Effect of sigma ligands on the cocaine-induced convulsions in mice.
771 *Polish Journal of Pharmacology* **51** 477–483.

772 Su T-P, Hayashi T, Maurice T, Buch S & Ruoho AE 2010 The sigma-1 receptor
773 chaperone as an inter-organelle signaling modulator. *Trends in Pharmacological*
774 *Sciences* **31** 557–566. (doi:10.1016/j.tips.2010.08.007)

775 Su TP, Su TC, Nakamura Y & Tsai SY 2016 The Sigma-1 Receptor as a Pluripotent
776 Modulator in Living Systems. *Trends in Pharmacological Sciences* **37** 262–278.
777 (doi:10.1016/j.tips.2016.01.003)

778 Sun H, Shi M, Zhang W, Zheng YM, Xu YZ, Shi JJ, Liu T, Gunosewoyo H, Pang T,
779 Gao ZB *et al.* 2016 Development of Novel Alkoxyisoxazoles as Sigma-1 Receptor
780 Antagonists with Antinociceptive Efficacy. *Journal of Medicinal Chemistry* **59**
781 6329–6343. (doi:10.1021/acs.jmedchem.6b00571)

782 Uchimura N & North RA 1990 Actions of cocaine on rat nucleus accumbens neurones
783 in vitro. *British Journal of Pharmacology* **99** 736–740.

784 Ujike H, Kuroda S & Otsuki S 1996 σ receptor antagonists block the development of
785 sensitization to cocaine. *European Journal of Pharmacology* **296** 123–128.
786 (doi:10.1016/0014-2999(95)00693-1)

787 Valjent E, Pagès C, Hervé D, Girault JA & Caboche J 2004 Addictive and non-
788 addictive drugs induce distinct and specific patterns of ERK activation in mouse
789 brain. *European Journal of Neuroscience* **19** 1826–1836. (doi:10.1111/j.1460-
790 9568.2004.03278.x)

791 Valjent E, Corbille A-G, Bertran-Gonzalez J, Herve D & Girault J-A 2006 Inhibition of
792 ERK pathway or protein synthesis during reexposure to drugs of abuse erases
793 previously learned place preference. *Proceedings of the National Academy of*
794 *Sciences* **103** 2932–2937. (doi:10.1073/pnas.0511030103)

795 Vaupel DB 1983 Naltrexone fails to antagonize the sigma effects of PCP and SKF
796 10,047 in the dog. *European Journal of Pharmacology* **92** 269–274.

797 Witkin JM, Terry P, Menkel M, Hickey P, Pontecorvo M, Ferkany J & Katz JL 1993
798 Effects of the selective sigma receptor ligand, 6-[6-(4-
799 hydroxypiperidinyl)hexyloxy]-3-methylflavone (NPC 16377), on behavioral and
800 toxic effects of cocaine. *J Pharmacol Exp Ther* **266** 473–482.

801 Wu Z & Bowen WD 2008 Role of sigma-1 receptor C-terminal segment in inositol
802 1,4,5-trisphosphate receptor activation: constitutive enhancement of calcium
803 signaling in MCF-7 tumor cells. *The Journal of Biological Chemistry* **283** 28198–
804 28215. (doi:10.1074/jbc.M802099200)

805 Yano H, Bonifazi A, Xu M, Guthrie DA, Schneck SN, Abramyan AM, Fant AD, Hong
806 WC, Newman AH & Shi L 2018 Pharmacological profiling of sigma 1 receptor
807 ligands by novel receptor homomer assays. *Neuropharmacology* **133** 264–275.
808 (doi:10.1016/j.neuropharm.2018.01.042)

809 Zhang LLL, Huang L, Lu K, Liu Y, Tu G, Zhu M, Ying L, Zhao J, Liu N, Guo F *et al.*
810 2017 Cocaine-induced synaptic structural modification is differentially regulated
811 by dopamine D1 and D3 receptors-mediated signaling pathways. *Addiction*

Biology **22** 1842–1855. (doi:10.1111/adb.12462)

Legend to figures

Figure 1. Endocrine actions of ghrelin in different body organs. Ghrelin is produced in the stomach (yellow) and exerts actions in different parts of the mammalian body. Examples of organs and actions are in green circles.

Figure 2. Mechanisms of ghrelin receptor-mediated cocaine-suppression of appetite. In the absence of cocaine, the peptide hormone ghrelin arrives to the CNS and activates GHS-R1a in CNS neurons to engage Gi signaling and the MAPK pathway (left). In the presence of cocaine activation of σ_1 produces conformational changes in GHS-R1a (see Aguinaga et al., 2018) that blocks any signaling originating at the ghrelin receptor (right). The chemical structure of cocaine is shown near the coca leaf.

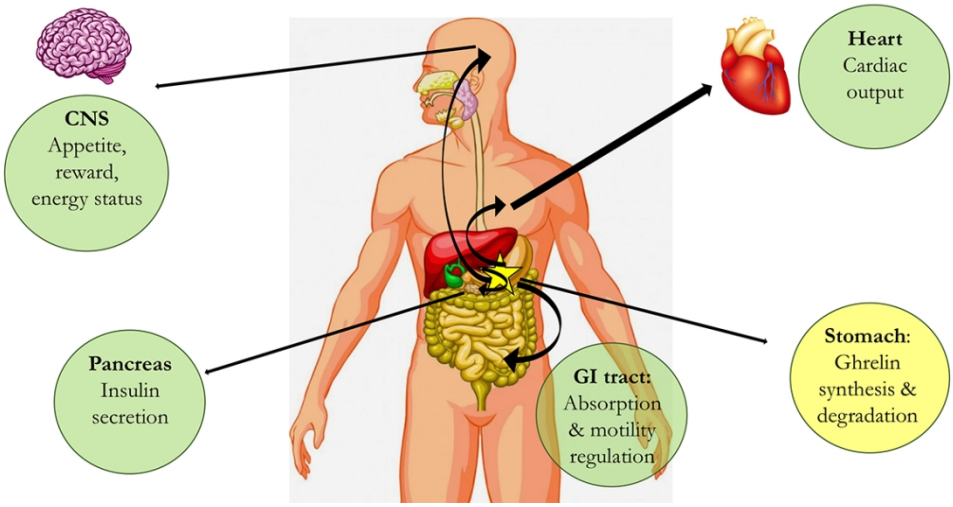

Figure 1. Endocrine actions of ghrelin in different body organs. Ghrelin is produced in the stomach (yellow) and exerts actions in different parts of the mammalian body. Examples of organs and actions are in green circles.

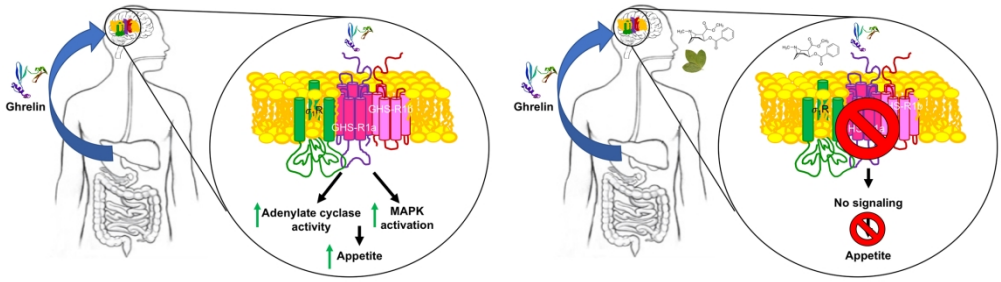

Figure 2. Mechanisms of ghrelin receptor-mediated cocaine-suppression of appetite. In the absence of cocaine, the peptide hormone ghrelin arrives to the CNS and activates GHS-R1a in CNS neurons to engage Gi signaling and the MAPK pathway (left). In the presence of cocaine activation of □1 produces conformational changes in GHS-R1a (see Aguinaga et al., 2018) that blocks any signaling originating at the ghrelin receptor (right). The chemical structure of cocaine is shown near the coca leaf.