Cocaine Effects on Dopaminergic Transmission Depend on a Balance between Sigma-1 and Sigma-2 Receptor Expression

David Aguinaga1,2, Mireia Medrano1,2, Ignacio Vega-Quiroga3, Katia Gysling3, Enric I. Canela1,2, Gemma Navarro1,4* and Rafael Franco1,2*

1 Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain, 2 Department of Biochemistry and Molecular Biomedicine, School of Biology, Universitat de Barcelona, Barcelona, Spain, 3 Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile, 4 Department of Biochemistry and Physiology, Faculty of Pharmacy, Universitat de Barcelona, Barcelona, Spain

Sigma σ1 and σ2 receptors are targets of cocaine. Despite sharing a similar name, the two receptors are structurally unrelated and their physiological role is unknown. Cocaine increases the level of dopamine, a key neurotransmitter in CNS motor control and reward areas. While the drug also affects dopaminergic signaling by allosteric modulations exerted by σ1R interacting with dopamine D1 and D2 receptors, the potential regulation of dopaminergic transmission by σ2R is also unknown. We here demonstrate that σ2R may form heteroreceptor complexes with D1 but not with D2 receptors. Remarkably, σ1, σ2, and D1 receptors may form heterotrimers with particular signaling properties. Determination of cAMP levels, MAP kinase activation and label-free assays demonstrate allosteric interactions within the trimer. Importantly, the presence of σ2R ligands increase cAMP signaling whereas reduce MAP kinase activation. These effects, which are opposite to those exerted via σ1R, suggest that the D1 receptor-mediated signaling depends on the degree of trimer formation and the differential balance of sigma receptor and heteroreceptor expression in acute versus chronic cocaine consumption. Although the physiological role is unknown, the heteroreceptor complex formed by σ1, σ2, and D1 receptors arise as relevant to convey the cocaine actions on motor control and reward circuits and as a key factor in acquisition of the addictive habit.

Keywords: acute, addiction, cAMP, chronic, dopamine D1 and D2 receptors, ERK1/2 phosphorylation, label-free, signaling

INTRODUCTION

In advanced societies cocaine addiction is an important health and socio-economic problem. Cocaine use begins recreationally and the seeking behavior is based on a feeling of general well-being. Drug addiction is the result of plastic changes in areas of the brain that have dopamine as the main neurotransmitter, particularly in the ventral tegmental area (VTA) (see Lüscher, 2013 and references therein). The main consequence of cocaine consumption in the central nervous system (CNS) is an increase in interneuronal dopamine levels, which is not limited to VTA but extends...
to other structures, such as the basal ganglia (Wise, 1984; Bradberry, 2008). It was thought that the inhibition of dopamine transporters was at the root of all the effects caused by this drug of abuse. However, there is strong evidence showing that cocaine exerts effects by a direct interaction with sigma receptors. Two different sigma receptors have been identified that are functionally and structurally unrelated. Although endogenous ligands are not known and the physiological function of sigma receptors is unclear, these receptors share the ability to bind cocaine. On the one hand, sigma-1 receptor ($\sigma_1$R) is a chaperone that spans once the membrane bilayer and whose recently reported structure consists of a homotrimer (Schmidt et al., 2016). On the other hand, sigma-2 receptor ($\sigma_2$R) was identified as a member of the family of membrane-associated progesterone receptors; apart from $\sigma_2$R (PGRMC1), three other human members are identified: PGRMC2, neudefrin, and neudesin. They are haem proteins displaying a cytochrome $b_5$-fold domain. While $\sigma_2$R dimerization affects proliferation and chemoresistance in tumor/metastasis in vitro models and xenograft-based tumor/metastasis models, the mode of action in the periphery and the CNS are virtually unknown (Kabe et al., 2016; reviewed in Cahill, 2017). The interaction of $\sigma_1$R with dopamine receptors and the relevant role that $\sigma_1$R exerts on the modulation of dopaminergic signaling by cocaine has been reported. In contrast, no study has been undertaken to know whether the binding of cocaine to $\sigma_2$R results in dopaminergic regulation.

The role of $\sigma_1$R as relevant target of cocaine was suspected due to the moderate affinity of drug binding to the receptor (Matsumoto et al., 2003; Hayashi and Su, 2005). Therefore, it seems that the "physiologically" relevant concentrations of cocaine can both inhibit the uptake of dopamine and activate $\sigma_1$R. $\sigma_1$R-cocaine interaction intervenes in the triggering of locomotor and convulsive actions of the drug (Menkel et al., 1991; Matsumoto et al., 2001a,b, 2002; Barr et al., 2015). In addition, synthetic drugs that act as $\sigma_1$R agonists and antagonists, respectively, potentiate (Matsumoto et al., 2002, 2003) and reduce (Matsumoto et al., 2004) cocaine actions. More recent studies have identified in both heterologous expression systems and natural sources an interaction between $\sigma_1$R and dopamine receptors (Navarro et al., 2010; Moreno et al., 2014; Borroto-Escuela et al., 2017). Accordingly, it has been suggested that dual antagonism of $\sigma_1$R and inhibition of the dopamine DAT transporter can effectively block cocaine self-administration (Katz et al., 2016). Matsumoto et al. (2007) reported that treatment with synthetic drugs that act on $\sigma_2$R attenuates cocaine-derived behavior in mice. Although the selectivity of the compounds was poor, 1 year later, Mésangeau et al. (2008) designed an approach for converting selective $\sigma_1$R ligands into $\sigma_2$R selective ligands that, importantly, showed anti-cocaine activity. Furthermore, it has been observed that treatment with $\sigma_2$R antagonists counteract locomotor stimulation induced by cocaine in mice (Lever et al., 2014; Guo and Zhen, 2015).

An important physiological consequence of cocaine ingestion is an increase in motor activity, which is controlled by basal-ganglia brain circuits. Motor control is exerted by the direct and indirect pathways of the basal ganglia and associated nuclei. Of the five types of dopamine receptors, the D$_1$ (D$_1$R) is enriched in the direct pathway, while the D$_2$ (D$_2$R) is enriched in the indirect route. The balance of the dopaminergic input in the two circuits results in fine-tuning motor control. The locomotor hyperactivity resulting from cocaine use probably reflects a lack of balance in these two routes. The objective of this work was to investigate how the binding of cocaine to $\sigma_2$R affects dopaminergic signaling mediated by D$_1$R and/or D$_2$R. We first investigated whether $\sigma_2$R interacts with D$_1$R or with D$_2$R and, subsequently, we observed how cocaine could affect in a $\sigma_1$R-independent but $\sigma_2$R-dependent fashion the signal transduction triggered by agonist activation of D$_1$R but not of D$_2$R.

**RESULTS**

$\sigma_2$R May Form Complexes with Dopamine D$_1$ But Not with Dopamine D$_2$ Receptors

Two different sigma receptors have been described, the non-opioid receptor, $\sigma_1$R, and the PGRMC1 protein, also known as $\sigma_2$. Despite the endogenous ligands are not known, the two sigma receptors may bind cocaine. While recent studies have demonstrated that $\sigma_1$R is involved in cocaine modulation of dopamine receptor function, a similar study on $\sigma_2$R-mediated modulation of dopaminergic signaling is lacking. We first evaluated in a heterologous expression system whether $\sigma_2$R may colocalize with dopamine receptors at the plasma membrane. Immunocytochemistry assays were undertaken in HEK-293T cells expressing $\sigma_2$R fused to RLuc and either dopamine D$_1$R fused to YFP or dopamine D$_2$R fused to YFP. The $\sigma_2$R expression was identified by a specific antibody against RLuc protein and a secondary Cy3 antibody, while dopamine receptor-YFP expression was identified by its own fluorescence. D$_1$R (green) was detectable at the plasma membrane level while $\sigma_2$R (red) was expressed both in intracellular structures and at the plasma membrane, where it colocalized (yellow) with D$_1$R (Figure 1A, left images). When a similar experiment was developed with D$_2$R, similar results were obtained indicating that D$_2$R and $\sigma_2$R colocalize at the cell surface (Figure 1A, right images). When the immunocytochemical assays were performed in cells pretreated with 30 $\mu$M cocaine for 30 min, the level of colocalization between $\sigma_2$R and D$_1$R or D$_2$R was similar, indicating that cocaine pretreatment did not affect neither cell surface expression of $\sigma_2$R, D$_1$R or D$_2$R nor receptor colocalization. Next, we determined whether $\sigma_2$R may form heteromer complexes with dopamine D$_1$ or D$_2$ receptors. For this purpose, we took advantage of energy transfer assays and in situ proximity ligation assay (PLA), which allows the identification of close proximity between two proteins (<17 nm) (Borroto-Escuela et al., 2011; Trifilieff et al., 2011). For PLA, HEK-293T cells expressing $\sigma_2$R and either D$_1$R or D$_2$R were treated with specific primary antibodies against $\sigma_2$R and against each of the dopamine receptors. Interestingly, the red punctuated signal around Hoechst-stained nuclei was much higher for D$_1$R and $\sigma_2$R than for D$_2$R and $\sigma_3$R (82 versus 27% of labeled cells) (Figure 1B). Finally,
we developed bioluminescence energy transfer assays in HEK-293T cells transfected with cDNAs for $\sigma_1$R-Rluc or $\sigma_2$R-Rluc and increasing amounts of cDNA for $\sigma_2$R-YFP. Interestingly, a saturable BRET curve was obtained ($\text{BRET}_{\text{max}} = 50 \pm 3$, $\text{BRET}_{\text{50}} = 190 \pm 40$) (Figure 2A) indicating a specific interaction between $D_1$R-$\sigma_2$R in contrast, a linear signal was obtained between $D_2$R-$\sigma_2$R (Figure 2B) suggesting a lack of interaction between them. When the same experiments were undertaken in cells treated with cocaine, similar results were obtained for the $D_1$R-Rluc/$\sigma_2$R-YFP donor/acceptor pair ($\text{BRET}_{\text{max}} = 82 \pm 10$, $\text{BRET}_{\text{50}} = 680 \pm 200$), indicating that cocaine did not significantly affect the interaction (Figure 2B).

**Dopamine $D_1$R, $\sigma_1$R, and $\sigma_2$R May Form Heterotrimeric Complexes**

Dopamine $D_1$ and $\sigma_1$ receptors may form heteromeric complexes in HEK-293T cells (Navarro et al., 2010). To confirm whether in our experimental conditions $D_1$R-Rluc may act as a donor of $\sigma_1$R-YFP, BRET experiments undertaken in cotransfected HEK-293T cells provided a saturable curve thus indicating the interaction between $\sigma_1$R and $D_1$R (Figure 2C). We then hypothesized that $\sigma_1$R and $\sigma_2$R could be interacting together. Accordingly, BRET assays were performed in HEK-293T cells expressing a constant amount of $\sigma_1$R-Rluc and increasing amounts of $\sigma_2$R-YFP. The unspecific linear signal obtained (Figure 2D) suggested that no interaction was occurring between the two sigma receptors. We then performed assays to investigate whether $\sigma_1$R and $\sigma_2$R competed for the binding to $D_1$R. BRET experiments were then developed in HEK-293T cells expressing a constant amount of $\sigma_1$R-Rluc and $D_1$R-YFP and increasing amounts of non-fused $\sigma_2$R. The results indicated that $\sigma_2$R was not able to compete with $\sigma_1$R for heteromer formation since the energy transfer between donor and acceptor was not altered (Figure 2E). When a similar experiment was performed expressing a constant amount of $D_1$R-Rluc and of $\sigma_2$R-YFP and increasing amounts of non-fused $\sigma_2$R, the results indicated that low expression levels of $\sigma_2$R increased BRET signals; however, higher expression levels of $\sigma_1$R were able to displace $\sigma_2$R out of the heteromer, as reflected by a significant decrease in BRET signal (Figure 2F). This result could reflect the formation of $D_1$R-$\sigma_1$R-$\sigma_2$R heterotrimer complexes, where the interaction of $\sigma_1$R to the $\sigma_2$R-D1R complex could create a structural change in turn leading to increasing the energy transfer between Rluc and YFP. To confirm this possibility, sequential resonance energy transfer (SRET) assays, which permits detection of trimers (Carriba et al., 2008), were developed in HEK-293T cells.
Our next aim was to characterize the functionality of the \( \sigma_1 \)-\( \sigma_2 \) receptor fused to RFP thus confirming the specificity of the triple \( \sigma_1 \)-\( \sigma_2 \)-\( \sigma_1 \) was performed by substituting complexes was occurring (Figures 2G,H). The negative control was performed by substituting \( \sigma_2 \)-RFP by the cannabinoid CB1 receptor fused to RFP thus confirming the specificity of the triple \( \sigma_1 \)-\( \sigma_2 \)-\( \sigma_1 \) interaction (Figure 2G).

\( \sigma_2 \) Activation Blocks Dopamine \( \sigma_1 \) Signaling

Our next aim was to characterize the functionality of the \( \sigma_1 \)-\( D_1 \)-\( \sigma_2 \)-\( \sigma_1 \) heterotrimer structure in HEK-293T cells treated with cocaine. It should be noted that \( \sigma_1 \) (Navarro et al., 2010) and \( \sigma_2 \) (Johannessen et al., 2011) are endogenously expressed in HEK-293T cells; consequently, we used a siRNA approach to silence \( \sigma_1 \) or \( \sigma_2 \) expression thus impeding heterotrimer formation. When HEK-293T cells were transfected with \( D_1 \) and siRNA for \( \sigma_1 \), SKF-81297-induced a significant increase in cAMP levels, that was inhibited by pretreatment with cocaine or with the \( \sigma_2 \) agonist, PB-28, indicating that cocaine decreases \( D_1 \)-mediated cAMP signaling function through its binding to \( \sigma_2 \) (Figure 3A). When HEK-293T cells were transfected with \( D_1 \) and siRNA for \( \sigma_2 \), the results indicated that cocainepretreatment potentiated agonist-induced cAMP levels, which was evidence of cocaine action upon binding to the \( \sigma_1 \) (Figure 3B). The next set of results is consistent with a reciprocal modulation of signaling mediated by cocaine binding to \( \sigma_2 \) and \( \sigma_1 \); while cocaine via \( \sigma_1 \) positively modulates cAMP levels, it inhibits cAMP signaling via \( \sigma_2 \). Accordingly, no effect of cocaine was observed in HEK-293T expressing \( D_1 \) and the two endogenous sigma receptors (Figure 3C). The lack of modulation exerted by cocaine upon simultaneous binding to both \( \sigma_1 \) and \( \sigma_2 \) likely reflects a balance which would, in a physiological set-up, depend on the relative expression of the two sigma receptors. In fact, when HEK-293T cells were transfected with \( D_1 \) and both siRNA for \( \sigma_1 \) and \( \sigma_2 \), cocaine or the specific \( \sigma_2 \) agonist, PB-28, had no effect, indicating that cocaine modulation over \( D_1 \) depends on \( \sigma_1 \) and \( \sigma_2 \) expression (Figure 3D).
We next investigated whether cocaine binding to σ2R receptors could still modulate D2R-mediated signaling. HEK-293T cells transfected with cDNAs for D2R and siRNA for σ1R, responded to the selective-D2R agonist, sumanorile. In these cells the G1-mediated decrease of forskolin-induced cAMP accumulation due to G1 coupling was not affected by cocaine pretreatment (Figure 3E). These results agree with the lack of interaction between σR and dopamine D2R (see Figure 2B). As a control, we confirmed that when the σ1R-cocaine modulation over D2R was assayed, i.e., silencing σ2R expression, cocaine was able to block the sumanorile-induced effect (Figure 3F).

These results agree with those in Navarro et al. (2013) in the sense that they reflect the consequence of a physical interaction between σ1R and D2R receptors. In agreement with this hypothesis, HEK-293T cells expressing D2R and endogenous sigma receptors behaved as cells in which the σ2R was silenced (Figure 3G). As a further control, HEK-293T cells treated with siRNAs to silence both sigma receptors showed no modulation by cocaine over D2R-mediated signaling (Figure 3H), thus reinforcing the idea that cocaine effect over D2R depends on σ1R expression.

**σ2R Activation Potentiates Dopamine D1R MAP Kinase Phosphorylation**

To further understand the cocaine effect over D1R function, MAP kinase signaling was evaluated in HEK-293T cells transfected with cDNAs for D1R and siRNA for either σ1R or σ2R. In cells expressing D1R with silenced σ1R, i.e., expressing D1R and σ2R (Figure 4A), cocaine pretreatment increased agonist (SFK-81297)-induced ERK1/2 phosphorylation, while in cells with silenced σ2R, i.e., expressing D1R and σ1R (Figure 4B), cocaine decreased agonist-induced ERK1/2 phosphorylation. These results are evidence of potentiation by cocaine-σ2R of MAP kinase signaling, and potentiation by cocaine-σ1R of G-protein dependent signaling. In cells expressing D1R and the two sigma receptors, no effect of cocaine pretreatment on pERK1/2 levels was observed, in agreement with the above-described balance resulting from reciprocal sigma-receptor-mediated cocaine effects (Figure 4C). As a further control, cocaine did not alter the SKF-81297-induced ERK1/2 phosphorylation in HEK-293T cells expressing D1R and with silenced sigma receptors (Figure 4D). A similar experimental design was used to undertake dynamic mass redistribution (DMR) assays. DMR is a label-free technique useful to investigate the activation of G-protein coupled receptors (Grundmann and Kostenis, 2015; Medrano et al., 2017). On the one hand, in cells expressing D1R and σ2R, cocaine blocked SKF-81297-induced increase in the DMR signal in a similar way as the selective σ2R ligand, PB-28, did (Figure 4E). On the other hand, the SKF-81297 effect was potentiated by cocaine pretreatment in cells expressing D1R and σ1R (Figure 4F). Once more, cocaine modulation on D1R-agonist-induced effects was not found in cells expressing D1R and both sigma receptors (Figure 4G). As DMR in cells expressing D1R mainly reflects Gs-coupling (Kebig et al., 2009;
Schröder et al., 2009; Hamamoto et al., 2015), these results are similar to those obtained in cAMP read-outs. Another control was performed to show that pretreatment with the σ2R selective agonist, PB-28, did not result in any signal modulation in cells expressing D1R but silenced σ1R and σ2R expression (Figure 4H).

**σ2R Activation Blocks Dopamine D1R-Mediated Signaling in Primary Cultures of Striatal Neurons**

A proximity ligation assay (PLA) was used to determine in primary cultures of striatal neurons whether D1R-σ2R complex expression was affected by cocaine pretreatment. Consequently, specific antibodies against D1R and σ2R were used in neurons treated or not with cocaine for 30 min (Figure 5A). 32% of cells showed punctuated staining (with 2.2 red spots/cell containing spots) surrounding Hoechst-stained nuclei (Figure 5B). These results indicate the occurrence of D1-σ2 heteroreceptor complexes in striatal primary cultures of neurons. A control done in the absence of primary antibodies led to 18% of labeled cells (with 1.2 red spots/cell containing spots). The percentage of positive cells after a 30-min treatment with cocaine was around 30 (with 2 red spots/cell containing spots) (Figure 5B). Thus, cocaine pretreatment did not significantly alter D1R-σ2R complex formation. When PLA was developed to detect D2R and σ2R complexes, the results (19% with 1.3 red spots/cell containing spots) were similar to those in the negative control (20% with 1.4 red spots/cell containing spots), i.e., no evidence of heteroreceptor formation was obtained. Pretreatment with cocaine did not lead to the appearance of heteromer complexes formed by D2R and σ2R (Figure 5B). These results agree with the BRET assays that did not find sign of interaction between the D2R-Rluc and σ2R-YFP or between the D1R-Rluc and σ2R-YFP pair.

To demonstrate the effect of cocaine over D1R-mediated signaling in a more physiological environment, we analyzed cAMP and MAP kinase signaling pathways in primary cultures of striatal neurons. As striatal neurons express the two sigma receptors, the siRNA approach was used to silence sigma receptor expression. On the one hand, in neurons transfected with siRNA for σ1R, and consequently expressing D1R and σ2R, cocaine and PB-28 led to a decrease in agonist-induced cAMP levels and to an enhancement in MAP kinase signaling (Figures 5C,G). On the other hand, in neurons transfected with the siRNA for σ2R, and consequently expressing D1R and σ1R, cocaine but not PB-28 induced an increase in the cAMP signal and a decrease in the ERK1/2 phosphorylation signal (Figures 5D,H). Most of these results agree with those obtained in the heterologous system. However, in striatal neurons...
expressing Δ1R and both sigma receptors, cocaine treatment led to a net effect that showed predominance of σ1R- versus σ2R-mediated modulation (Figures 5E, I). These findings could be due to a higher expression of σ1R-Δ1R complexes versus σ2R-Δ1R but they may also result from the lower affinity of the cocaine/σ2R binding (Lever et al., 2016). Finally, another control was performed to show that pretreatment with the σ2R selective agonist, PB-28, or with cocaine, did not result in any signal modulation in cells expressing Δ1R but silenced σ1R and σ2R expression (Figures 5E, J).

**Δ1R-Mediated Signaling Is Modulated by σ1R in Acute and by σ2R in Chronic Conditions**

*In situ* PLAs were performed to identify Δ1R-σ1R and Δ1R-σ2R heteroreceptor complexes in striatal sections from Sprague–Dawley rats receiving cocaine under acute or chronic regimes (see section “Materials and Methods”) (Figure 6A). When striatal sections of vehicle-treated animals were analyzed, it was observed that 38.5% of cells showed Δ1R-σ1R complexes with 2.5 red spots/cell containing spots, while only 25% of cells showed Δ1R-σ2R complexes with 2.1 dots/cell (Figure 6B). When Sprague–Dawley rats were acutely treated with cocaine, it was observed that both Δ1R-σ1R and Δ1R-σ2R complex expression increased. However, the Δ1R-σ1R complexes doubled its expression while Δ1R-σ2R complex expression suffered a slight increase (respectively, 54% of cells showed red spots with 4.5 spots/cell and 33% with 2.3 spots/cell). Interestingly, in the case of rats chronically treated with cocaine, the Δ1R-σ1R heteromer complex expression was not affected (34% of cells showed red spots with 2.4 spots/cell containing spots) compared to control animals, while the Δ1R-σ2R heteromer expression significantly increased (35% of cells containing spots with 3.4 spots/cell containing spots) (Figure 6B). These results indicate that acute cocaine treatment strongly increases Δ1R-σ1R complexes formation in striatal rat sections but chronic cocaine treatment only drives Δ1R-σ2R complex expression. Then, we questioned if the cocaine-induced alterations in Δ1R-σ1R and Δ1R-σ2R complex expression had signaling consequences. To do so, we analyzed SKF-81297-induced cAMP production in primary cultures of striatal neurons pretreated with vehicle or cocaine for
different times (from 0.5 h to 7 days). Interestingly, we observed that at short times SKF-81297-induced cAMP levels were further increased. In agreement with results in HEK-293 cells, cocaine binding to the σ1R induced a positive modulation over dopamine D1R-mediated signaling. When primary cultures of neurons were longer exposed to cocaine (1–7 days), SKF-81297-induced increase in cAMP levels was inhibited (Figure 6C). Taking into account the results in HEK-293 cells such effect seems associated to D1R-σ2R complex formation and to the ability of σ2R to counteract the SKF-81297-induced increases of cAMP. To check whether these interpretations were correct, i.e., if σ2R receptors were responsible of cocaine-induced modulations over D1R-mediated signaling, primary striatal neurons were transfected with siRNA specific for σ1R or σ2R. On the one hand, cocaine pretreatment (0.5 h to 7 days) blocked SKF-81297-induced accumulation of cAMP levels in primary cultures of neurons transfected with siRNA for σ1R, i.e., expressing D1R and σ2R (Figure 6D). On the other hand, 0.5 h and 2 h pretreatment of cocaine potentiated the SKF-81297-induced increases in cAMP levels in primary neurons transfected with siRNA for σ2R, i.e., expressing D1R and σ1R. However, longer periods of cocaine exposure (1–7 days) produced no effect (Figure 6E). These results suggest that in acute cocaine treatment D1R form heteromers mainly with σ1R, prevailing the D1R-σ1R-mediated signaling. In contrast, in the chronic situation, the increase of σ1R-D1R heteromer complex expression observed in acute conditions disappear but the increase in the D1R-σ2R complex expression is maintained, being the σ2R responsible of the...
cocaine modulation over D$_1$R, hence prevailing the D$_1$R-σ$_2$R-mediated signaling.

**DISCUSSION**

Sigma receptors are relevant in cocaine addiction, because binding of cocaine to these receptors modulates dopaminergic transmission. Although cocaine can bind to both σ$_1$R and σ$_2$R, they are not closely related and no common structural properties have been identified. With respect to the modulation of receptor-mediated signaling, a relevant difference is revealed by the formation of heteroreceptor complexes. On the one hand, σ$_1$R interacts with D$_1$ and D$_2$ dopamine receptors (Navarro et al., 2010; Moreno et al., 2014). Interestingly, we here report that σ$_2$R may form heteromeric complexes with D$_1$R but not with D$_2$R.

Information on PGRMC1/σ$_2$R expression in brain is partial. Intlekofer and Petersen (2011) confirmed data by Krebs et al. (2000) showing enrichment of the receptor in nuclei of the hypothalamus that are important for female reproduction. Petersen et al. (2013) in 2013, reviewed neuroanatomical data on the expression PGRMC1 and related proteins in CNS neuroendocrine nuclei. To our knowledge, information on expression in other neural regions is either absent or preliminary. Interestingly, a recently developed fluorescent probe tested in rat brain indicates that the receptor is more present in neurons than in glial cells (Zeng et al., 2016). Despite good *in vitro* properties, some of the radiolabeled probes that were developed for *in vivo* σ$_2$ receptor imaging have not reached the final objective (Abate et al., 2013; Selivanova et al., 2015). In contrast, recently reported 18F-labeled PET probes, with enhanced brain uptake and σ$_2$R selectivity (in mice), show promise for *in vivo* imaging of the receptor in the human brain (Wang et al., 2017). Surely these novel tools will be instrumental to achieve a more detailed mapping of the receptor in the CNS, specially in those areas in which dopamine receptors are expressed. There is, however, strong evidence of expression in the striatum; for instance, a recent report shows that receptor agonist regulate dopaminergic input into the striatum and the receptor is presynaptically expressed the *nucleus accumbens* (Klawonn et al., 2017). Furthermore, pioneering studies by Werling and colleagues showed σ$_2$R involvement in control of dopamine transporter activity in striatum (Derbez et al., 2002) and that the striatal receptor was a target of cocaine (Nuwayhid and Werling, 2006).

The results presented here and those already reported (Navarro et al., 2010, 2013) show that in equivalent experimental configurations, cocaine binding to σ$_1$R improves the accumulation of cAMP mediated by D$_1$R and inhibits MAP kinase signaling. Cocaine, via σ$_2$R, blocks D$_2$-mediated cAMP accumulation and enhances MAP kinase activation. Importantly, similar results were obtained in HEK-293T cells and primary neuronal cultures.

D$_1$R can form complexes and high- order heteromers by interacting simultaneously with the σ$_1$ and σ$_2$ receptors, σ$_1$R being able to displace σ$_2$R, but not vice versa. Navarro et al. (2010) reported an increase in the plasma membrane expression of σ$_1$R after acute exposure to cocaine. When increase in σ$_1$R levels in the plasma membrane occurs, σ$_2$R is displaced from the D$_1$R-σ$_2$R or D$_1$R-σ$_1$R-σ$_2$R heteroreceptor complexes. Such phenomenon results in increasing the amount of D$_1$-σ$_1$ heteroreceptors and D$_1$R signaling whereas, as reported by Navarro et al. (2013), reducing D$_2$R-mediated actions. However, in a longer exposure to cocaine, the signaling mediated by the dopamine D$_1$R fits more with that occurring via a D$_1$R-σ$_2$R functional unit. These data suggest that the initial cocaine-induced overexpression in the plasma membrane of the σ$_1$R is transient; once these levels decrease, due to internalization or other still unknown mechanisms, σ$_2$ is the predominant receptor forming heteromers with dopamine D$_1$R.

Motor control in the basal ganglia is achieved through a complex circuit composed of GABAergic neurons that contain mainly D$_1$R (direct pathway) and GABAergic neurons containing mostly D$_2$R (indirect pathway) (Grillner and Robertson, 2016). Fine motor control is achieved by a balance of dopaminergic signals, one via D$_1$ receptors, which are G$_i$ coupled, and another via D$_2$ receptors, which are G$_i$ coupled (Jenner, 1995; Gerfen, 2000). The deterioration of motor control by cocaine depends on the imbalance of the direct/indirect pathway, but the underlying mechanism remains unclear. Although the scenario is complex, cocaine is known to increase cAMP levels in cells expressing D$_1$R-σ$_1$R (Navarro et al., 2010). Therefore, cocaine seems to be increasing in the direct pathway the cAMP-dependent dopaminergic output, namely activation of protein kinase A and cAMP-regulated DARPP-32 phosphoprotein (Svenningsson et al., 2004). Through the same receptor (σ$_1$R), cocaine leads to a deterioration of the dopaminergic performance of the indirect route (Navarro et al., 2013). In addition to the imbalance resulting from these σ$_1$R-dependent effects, our results demonstrate that trimers of D$_1$, σ$_1$, and σ$_2$ receptors may be formed and that cocaine acting on these heteromers reduces the negative modulation exerted by the D$_1$R-σ$_1$R complexes.

The results here presented also show that the MAP kinase signaling pathway is particularly affected by the action of cocaine upon dopamine-sigma heteroreceptors. While in cells expressing the D$_1$R-σ$_1$R heteromer, cocaine decreased ERK1/2 phosphorylation, cocaine did the opposite in cells expressing the D$_1$R-σ$_2$R heteromer. It is known that ERKs are involved in the plastic changes induced by the consumption of drugs of abuse (Radwanska et al., 2005). In addition, the inhibition of ERK phosphorylation alters learned place-preference in a paradigm of drug-of-abuse consumption, whereas activation of ERK1/2 is necessary to establish the association between place preference and drug consumption (Valjent et al., 2006; Du et al., 2017). In this context, knocking down ERK1 has shown that enhanced ERK2 signaling and repeated exposure to the drug facilitate the plastic changes leading to drug addiction (Ferguson et al., 2006). It should be noted that the temporal pattern of MAP kinase activation in the mouse brain is differently induced by addictive or non-addictive drugs (Valjent et al., 2004). Interestingly, Zhang et al. (2017) have described that D$_1$ receptor antagonists alter in cocaine-treated mice the length of *nucleus accumbens* postsynaptic densities, i.e., cocaine-induced...
long-term plasticity; however, the mechanism underlying this phenomenon has not been described. According to the previous reports and to our results, it may be suggested that potentiation of MAP kinase pathway mediated by the $D_1R$-$\sigma_2R$ heteromer may be the mechanism by which the $\sigma_2R$ would induce long term neuronal plasticity. The predominant role of the $\sigma_1R$ in acute cocaine use shifts to a more relevant role of $\sigma_2R$ in the chronic condition leading to the establishment of addiction. In any case, the relative expression of the two receptors in a given neuron seems important in determining the fate of the cell when the drug of abuse is consumed.

In acute cocaine exposure, $\sigma_1R$ modulation of $D_1R$-mediated signaling prevails, but in longer exposures, there is a shift to regulation by $\sigma_2R$. Recently, Singer et al. (2017) have determined that neuronal plasticity initiate 2 h after cocaine exposure. The mechanism of action described in this paper cannot explain some of the results reported by Matsumoto et al. (2007) and Lever et al. (2014) who report that $\sigma_2R$ receptor antagonists block the effects of cocaine-induced hyperlomocomotion. It should be, however, noted that a recent report show benefits of a $\sigma_2R$ -selective agonist, siramenes, for decreasing cocaine effects via reduction of dopaminergic and glutamatergic input to the striatum (Klawonn et al., 2017). On the one hand, it is a reasonable assumption that $\sigma_1R$ is more involved in the regulation of $D_1R$ signaling at acute exposure. However, it remains to be determined whether some of the results reported on the impact of cocaine on locomotion are due to the use of non-selective ligands, that is, ligands that can bind to both sigma receptors and alter their function. Alternatively, it may happen that $\sigma_2R$ is also affecting the direct route in acute conditions. What our results undoubtedly indicate is that $\sigma_2R$ becomes the main player in conditions of chronic exposure to the drug. In summary, some of the addictive and motor actions of cocaine are the result of a balance between cocaine-$\sigma_1R$ versus cocaine-$\sigma_2R$ impact on activation of $D_1R$ and $D_2R$ (and $D_1R$-$D_2R$, see Perreault et al., 2016) in ad hoc CNS circuits.

MATERIALS AND METHODS

Reagents

Cocaine-chlorhydrate was provided by the Spanish Agencia del Medicamento (Ref. n°: 2003C00220), $\sigma_2R$ agonist, 1-Cyclohexyl-4-[3-(1,2,3,4-tetrahydro-5-methoxy-1-naphthalenyl)propyl)piperazine dihydrochloride (PB-28), $D_1R$ agonist (±)-6-Chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzapine hydrobromide (SKF-81297) and $D_2R$ agonist, sumanireole, were purchased from Tocris, Bristol, United Kingdom.

Fusion Proteins and Expression Vectors

cDNAs for human versions of $D_1R$, $D_2R$, $\sigma_1R$, or $\sigma_2R$ cloned into pcDNA3.1 were amplified without their stop codons using sense and antisense primers harboring: EcoRI and KpnI sites to subclone $D_1R$, $D_2R$, $\sigma_1R$, and $\sigma_2R$ in pcDNA3.1Rluc vector (pRluc-N1, PerkinElmer Life and Analytical Sciences, Wellesley, MA, United States) or HindIII and BamHI sites to clone $D_1R$, $D_2R$, $\sigma_1R$, and $\sigma_2R$ in pEYFP-N1 vector (enhanced yellow variant of GFP, Clontech), or EcoRI and BamHI sites to clone $\sigma_2R$ in a cherry-containing vector (pcDNA3.1Cherry). Amplified fragments were subcloned to be in-frame with restriction sites for pRluc-N1, pEYFP-N1, or pcDNA3.1Cherry vectors to provide plasmids that express proteins fused to Renilla Luciferase ($D_1R$-Rluc, $D_2R$-Rluc, $\sigma_1R$-Rluc, and $\sigma_2R$-Rluc), YFP ($D_1R$-YFP, $D_2R$-YFP, $\sigma_1R$-YFP, and $\sigma_2R$-YFP) or cherry ($\sigma_2R$-Cherry) at the C-terminal end.

Cell Lines and Transient Transfection

HEK-293T human embryonic kidney cells were grown at 37°C in a humid atmosphere with 5% CO$_2$ in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Thermo Fischer Scientific, Madrid, Spain) supplemented with 2 mM L-glutamine, 100 µl/ml sodium pyruvate, 100 U/ml penicillin/streptomycin, MEM Non-Essential Amino Acid Solution (1/100) and 5% (v/v) heat inactivated foetal bovine serum (FBS) (all supplements were from Invitrogen, Paisley, Scotland, United Kingdom). Cells were transiently transfected with constructs encoding for receptors, fusion proteins, and/or siRNAs by the polyethylenimine (PEI; Sigma–Aldrich, St. Louis, MO, United States) method. Transfected cells were incubated in serum-free medium that after 4 h was replaced by complete medium. Experiments were carried out 48 h later.

Neuronal Primary Cultures

Primary cultures of striatal neurons were obtained from 19-day embryos of Sprague–Dawley rats. Cells were isolated as described in Hardisky et al. (2013) and plated at a confluence of 40,000 cells/0.32 cm$^2$. Cells were maintained for 12 days in Neurobasal medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, and 2% (v/v) B27 supplement (Gibco) in 6-well plates. When indicated, cells were transiently transfected with the corresponding siRNA (3 µg plasmid siRNA per well) using the Lipofectamine™ 2000 (Invitrogen, Life Technologies, Darmstadt, Germany). Transfected cells were incubated in serum-free medium that after 4 h was replaced by complete medium. Experiments were carried out 48 h later.

Cocaine Treatment of Sprague–Dawley Rats

Male Sprague–Dawley rats weighing 200–220 g were selected for the experiments. Rats were kept in controlled environment with 12 h light-dark cycle at 21°C room temperature. Food and water were provided ad libitum. Experimental procedures were approved by the Bioethical Committee of the Faculty of Biological Sciences of the Pontificia Universidad Católica de Chile and follow the international guidelines (NIH Guide for the Care and Use of Laboratory Animals). Rats were housed and handled in colony for three days, and then were divided in two experimental groups: acute and chronic, with respective saline controls. Chronic cocaine administration consisted in two injections of cocaine (15 mg/kg, i.p.) per day for 14 days at 11:00 A.M. and 5:00 P.M., as described by Liu et al. (2005). Acute cocaine administration consisted of two injections of...
cocaine (15 mg/kg, i.p.) for only one day. The same protocol of administration was used in control animals receiving saline injections. Rats were sacrificed 17 h after the last saline or cocaine injection following the protocol of Liu et al. (2005). Cocaine HCl was donated by the National Institute on Drug Abuse (NIDA, United States).

**Immunocytochemistry**

HEK-293T cells were treated with 30 μM cocaine or vehicle for 30 min, then were washed with PBS, fixed in 4% paraformaldehyde for 15 min and washed with PBS containing 20 mM glycine to quench free aldehyde groups. After permeabilization with PBS-glycine buffer containing 0.2% Triton X-100 for 5 min, cells were blocked with PBS containing 1% bovine serum albumin (BSA) for 1 h at room temperature. D1R-YFP and D2R-YFP were detected by its own fluorescence (wavelength 530 nm), and σ2R-Rluc was stained using a primary anti-Rluc mouse monoclonal antibody (1/200, Millipore, CA, United States) for 1 h, washed and stained for another hour with the secondary Cy3-conjugated donkey anti-mouse antibody (1/200, Jackson Immunoresearch Laboratories, West Grove, PA, United States). Nuclei were stained with Hoechst (1/100, Sigma–Aldrich, St. Louis, MO, United States) and then samples were rinsed several times and mounted with Mowiol 30% (Calbiochem). Images were taken using a Leica SP2 confocal microscope (Leica Microsystems, Mannheim, Germany).

**Proximity Ligation Assay**

For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips.

Proximity Ligation Assay

For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips. For PLAs, HEK-293T cells or primary cultures of striatal neurons were grown on glass coverslips while cocaine-administered rat sections were mounted and dried directly on glass coverslips.
regression equation, assuming a single-phase saturation curve with GraphPad Prism software (GraphPad Software). The relative amount of BRET or SRET is given as a function of $100 \times$ the ratio between the fluorescence of the acceptor (YFP or cherry) and the luciferase activity of the donor (Rluc).

### CAMP Determination

CAMP levels were assayed with different forskolin concentrations and cell densities to select the most appropriate conditions of the assay, which were 0.5 $\mu$M forskolin and 5,000 HEK-293T cells or 7,500 neurons. Transfected HEK-293T cells or neurons were incubated in serum-free medium for 3 h before the experiment. Then, cells were placed in 384-well microplates in medium containing 50 $\mu$M zardaverine (Tocris Bioscience). Cells were then preincubated with vehicle, the $\sigma_2$R agonist, PB-28 (300 nM) or cocaine (30 $\mu$M) for 15 min, followed by dopaminergic stimulation with the D$_2$R agonist, SKF-81297 (200 nM), the D$_2$R agonist, sumanilore (500 $\mu$M) or vehicle. After another incubation period of 15 min, 0.5 $\mu$M forskolin or vehicle were added. Readings were performed 15 min later by the use of a homogeneous time-resolved fluorescence energy transfer (HTRF) method requiring the Lance Ultra CAMP kit (PerkinElmer) and fluorescence readings (at 635 nm) in a PHERAs ter Flagship microplate equipped with a time-resolved fluorescence optical module (BMG Labtech).

### ERK1/2 Phosphorylation

To determine ERK1/2 phosphorylation, 40,000 HEK-293T cells/well or 50,000 neurons/well were plated in transparent Deltalab 96-well plates and kept in the incubator for 48 h. The medium was substituted by serum-free DMEM medium for 2–4 h before initiating the experiment. Then, HEK-293T cells and striatal neurons were pretreated for 10 min at 25°C with vehicle, PB-28 (300 nM) or cocaine (30 $\mu$M) followed by the addition of 200 nM SKF-81297, the D$_2$R specific agonist. 10 min after activation, cells/neurons were placed on ice and washed twice with cold PBS before the addition of 30 $\mu$l of lysis buffer for 15 min. Supernatants (10 $\mu$l) were placed in white ProxiPlate 384-well microplates, and ERK1/2 phosphorylation was determined using the AlphaScreen®SureFire® kit (Perkin Elmer) and the EnSpire® Multimode Plate Reader (PerkinElmer, Waltham, MA, United States).

### Data Analysis

The data in graphs are the mean ± SEM. The test of Kolmogorov–Smirnov with the correction of Lilliefors was used to evaluate normal distribution and the test of Levene to evaluate the homogeneity of variance. Parametric statistic methods were used, because results in the different groups showed normality and homogeneity of variance. Significance was analyzed by one-way ANOVA, followed by Dunnett’s multiple comparison post hoc test. GraphPad Prism software version 5 was used for the statistical analysis. Significant differences were considered when $p < 0.05$.

### Author Contributions

GN, EC, and RF designed the experiments and directed the project. DA did many of the cell and molecular assays and did the statistics of the results in the laboratory of the University of Barcelona. KG designed the experiments to obtain cocaine-treated animals in the laboratory in Chile. MM and IV-Q administered drugs to animals, prepared the brain sections, and treated animals in the laboratory in Chile. MM and IV-Q performed the immunological-based histochemical assays. GN and RF wrote the first draft of the manuscript, which was further edited by DA, KG, EC, and IV-Q.

### Acknowledgments

This work was supported by a grant from the Spanish Ministry of Economy and Competitiveness (Ref. No. BFU2015-64405-R; it may include FEDER funds) and a FONDECYT Grant No. 1150244.

### References

Abate, C., Selivanova, S. V., Müller, A., Krämer, S. D., Schibli, R., Marrotto, R., et al. (2013). Development of 3,4-dihydroisoquinolin-1(2H)-one derivatives for the positron emission tomography (PET) imaging of $\sigma_2$ receptors. Eur. J. Med. Chem. 69, 920–930. doi: 10.1016/j.ejmech.2013.09.018

Barr, J. L., Deliu, E., Brailoiu, G. C., Zhao, P., Yan, G., Abood, M. E., et al. (2015). Mechanisms of activation of nucleus accumbens neurons by cocaine via Sigma-1 receptor–inositol 1,4,5-trisphosphate–transient receptor potential canonical channel pathways. Cell Calcium 58, 196–207. doi: 10.1016/j.ceca.2015.05.001

Borroto-Escuela, D. O., Narváez, M., Wydra, K., Pintsuk, J., Pinton, L., Jimenez-Beristain, A., et al. (2017). Cocaine self-administration specifically increases A2AR-D2R and D2R-Sigma1R heteroreceptor complexes in the rat nucleus accumbens shell. Relevance for cocaine use disorder. Pharmacol. Biochem. Behav. 155, 24–31. doi: 10.1016/j.pbb.2017.03.003
Borrotto-Escuela, D. O., Van Craenenbroeck, K., Romero-Fernandez, W., Guidolin, D., Woods, A. S., Rivera, A., et al. (2011). Dopamine D2 and D4 receptor heteromerization and its allosteric receptor-receptor interactions. Biochem. Biophys. Res. Commun. 404, 928–934. doi: 10.1016/j.bbrc.2010.12.083
Bradberry, C. W. (2008). Comparison of acute and chronic neurochemical effects of cocaine and cocaine cues in rhesus monkeys and rodents: focus on striatal and cortical dopamine systems. Rev. Neurosci. 19, 113–128. doi: 10.1531/REVNEURO.2008.19.2-3.113
Cahill, M. A. (2017). The evolutionary appearance of signaling motifs in PGRMC1. Biosci. Trends 11, 179–192. doi: 10.5582/btst.2017.01009
Carriba, P., Navarro, G., Ciruela, F., Ferré, S., Casadó, V., Agnati, L., et al. (2011). Distribution of mRNAs encoding classical progestin receptor, progesterone membrane components 1 and 2, and CREB phosphorylation in rats. J. Pharmacol. Exp. Ther. 337, 306–314. doi: 10.1124/jpet.110.19914
Du, Y., Du, L., Cao, J., Hölscher, C., Feng, Y., Su, H., et al. (2017). Levo-tetradehydromelatonin inhibits the acquisition of ketamine-induced conditioned place preference by regulating the expression of ERK and CREB phosphorylation in rats. Behav. Brain Res. 317, 367–373. doi: 10.1016/j.bbr.2016.10.001
Ferguson, S. M., Fasano, S., Yang, P., Brambilla, R., and Robinson, T. E. (2006). Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. Neuropharmacology 31, 2660–2668. doi: 10.1088/1356-3402.13001
Gerfen, C. R. (2000). Molecular effects of dopamine on striatal-projection pathways. Trends Neurosci. 23(Suppl. 10), S64–S70. doi: 10.1016/S1471-1931(00)00019-7
Grillner, S., and Robertson, B. (2016). The basal ganglia over 500 million years. Am. J. Physiol. Cell Physiol. 301, C328–C337. doi: 10.1152/ajpcell.00383.2010
Kebig, A., Kostenis, E., Mohr, K., and Mohr-Andrä, M. (2009). Collecting human data for the 12th IUPHAR workshop on G protein-coupled receptors: an overview. J. Recept. Signal Transduct. Res. 29, 140–145. doi: 10.1080/10799890903412278
Klawn, A. M., Nilsson, A., Räldberg, C. F., Lindström, S. H., Ericson, M., Granath, B., et al. (2017). The Sigma-2 receptor selective agonist sirmasine (Lu 28-179) decreases cocaine-reinforced pavlovian learning and alters glutamatergic and dopaminergic input to the striatum. Front. Pharmacol. 8:714. doi: 10.3389/fphar.2017.00714
Krebs, C. J., Jarvis, E. D., Chan, J., Lydon, J. P., Ogawa, S., and Pfaff, D. W. (2000). A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors. Proc. Natl. Acad. Sci. U.S.A. 97, 12816–12821. doi: 10.1073/pnas.97.23.12816
Lever, J. R., Ferguson-cantrell, E. A., Watkinson, L. D., Carmack, T. L., Lord, S. A., Dong, X. U., et al. (2016). Cocaine occupancy of Sigma1 receptors and dopamine transporters in mice. Synapse 70, 98–111. doi: 10.1002/syn.2177
Lever, J. R., Miller, D. K., Green, R. L., Ferguson-Cantrell, E. A., Watkinson, L. D., Carmack, T. L., et al. (2014). A selective Sigma-2 receptor ligand antagonizes cocaine-induced hyperlocomotion in mice. Synapse 68, 73–84. doi: 10.1002/syn.21717
Liu, J., Yu, B., Orozco-Cabal, L., Grigoriadis, D. E., Rivier, J., Vale, W. W., et al. (2005). Chronic cocaine administration switches corticotropin-releasing factor2 receptor-mediated depression to facilitation of glutamatergic transmission in the lateral septum. J. Neurosci. 25, 577–583. doi: 10.1523/JNEUROSCI.496-04.2005
Lüscher, C. (2013). Cocaine-evoked synaptic plasticity of excitatory transmission in the ventral tegmental area. Cold Spring Harb. Perspect. Med. 3:a01213. doi: 10.1101/cshperspect.a01213
Matsumoto, R. R., Gilmore, D. L., Poub, B., Bowen, W. D., Williams, W., Kauras, et al. (2004). Novel analogs of the Sigma receptor ligand B1008 attenuate cocaine-induced toxicity in mice. Eur. J. Pharmacol. 492, 21–26. doi: 10.1016/j.ejphar.2004.03.037
Matsumoto, R. R., Hewett, K. L., Poub, B., Bowen, W. D., Husbands, S. M., Cao, J. J., et al. (2001a). Rimcazole analogs attenuate the convulsive effects of cocaine: correlation with binding to Sigma receptors rather than dopamine transporters. Neuropharmacology 41, 878–886.
Matsumoto, R. R., Liu, Y., Lerner, M., Howard, E. W., and Brackett, D. J. (2003). Sigma receptors: potential medications development target for anti-cocaine agents. Eur. J. Pharmacol. 469, 1–12. doi: 10.1016/S0014-2999(03)01723-0
Matsumoto, R. R., McCracken, K. A., Friedman, M. J., Poub, B., De Costa, B. R., and Bowen, W. D. (2001b). Conformationally restricted analogs of B1008 and an antisense oligodeoxynucleotide targeting Sigma1 receptors produce anti-cocaine effects in mice. Eur. J. Pharmacol. 419, 163–174.
Matsumoto, R. R., McCracken, K. A., Poub, B., Zhang, Y., and Bowen, W. D. (2002). Involvement of Sigma receptors in the behavioral effects of cocaine: evidence from novel ligands and antisense oligodeoxynucleotides. Neuropharmacology 42, 1043–1055. doi: 10.1016/S0028-3908(02)00056-4
Matsumoto, R. R., Poub, B., Mack, A. L., Daniels, A., and Coop, A. (2007). Effects of UMB24 and (+/-)-SM 21, putative Sigma2-prefering antagonists, on behavioral toxic and stimulant effects of cocaine in mice. Pharmacol. Biochem. Behav. 86, 86–91. doi: 10.1016/j.pbb.2006.12.011
Medrano, M., Agúinaga, D., Reyes-Resina, I., Canela, E. L., Mallol, J., Navarro, G., et al. (2017). Orexin A/Hypocretin modulates leptin receptor-mediated signaling by allosteric modulations mediated by the ghrelin ghb-r1a receptor in hypothalamic neurons. Mol. Neurobiol. doi: 10.1007/s12035-017-0670-8 [Epub ahead of print].
Menkel, M., Terry, P., Pontecorvo, M., Katz, J. L., and Wittkin, J. M. (1991). Selective Sigma ligands block stimulant effects of cocaine. Eur. J. Pharmacol. 201, 251–252. doi: 10.1016/0014-2999(91)90355-T
Mésangeau, C., Narayanan, S., Green, A. M., Shaikh, J., Kaushal, N., Viard, et al. (2008). Conversion of a highly selective Sigma-1 receptor ligand to Sigma-2 receptor preferring ligands with anticocaine activity. J. Med. Chem. 51, 3545–3558. doi: 10.1021/jm071357m
Moreno, E., Moreno-Delgado, D., Navarro, G., Hoffmann, H. M., Fuentes, S., Rossell-Vilar, S., et al. (2014). Cocaine disrupts histamine H3 receptor modulation of dopamine D1 receptor signaling: σ1-D3-H3 receptor complexes as key targets for reducing cocaine’s effects. J. Neurosci. 34, 3545–3558. doi: 10.1523/JNEUROSCI.4147-13.2014
Navarro, G., Moreno, E., Aymerich, M., Marcellino, D., McCormick, P. J., Mallol, J., et al. (2010). Direct involvement of σ-1 receptors in the dopamine...
D1 receptor-mediated effects of cocaine. Proc. Natl. Acad. Sci. U.S.A. 107, 18676–18681. doi: 10.1073/pnas.100891107
Navarro, G., Moreno, E., Bonaventura, J., Brugarolas, M., Farré, D., Aguinaga, D., 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
Nwokayid, S. J., and Werling, L. L. (2006). Sigma2 (σ2) receptors as a target for cocaine action in the rat striatum. Eur. J. Pharmacol. 535, 98–103. doi: 10.1016/j.ejphar.2005.12.077
Perreault, M. L., Hasbi, A., Shen, M. Y. F., Fan, T., Navarro, G., Fletcher, P. J., et al. (2016). Disruption of a dopamine receptor complex amplifies the actions of cocaine. Eur. Neuropsychopharmacol. 26, 1366–1377. doi: 10.1016/j.euroneuro.2016.07.008
Petersen, S. L., Intlekofer, K. A., Moura-Conlon, P. J., Brewer, D. N., Del Pino Sans, J., and Lopez, J. A. (2013). Nonclassical progesterone signalling molecules in the nervous system. J. Neuroendocrinol. 25, 991–1001. doi: 10.1111/jne.12060
Radwanska, K., Caboche, J., and Kaczmarek, L. (2005). Extracellular signal-regulated kinases (ERKs) modulate cocaine-induced gene expression in the mouse amygdala. Eur. J. Neurosci. 22, 939–948. doi: 10.1111/j.1460-9586.2005.04286.x
Schmidt, H. R., Zheng, S., Guppinar, E., Koehl, A., Manglik, A., and Kruse, A. C. (2016). Crystal structure of the human σ1 receptor. Nature 532, 527–530. doi: 10.1038/nature17391
Schröder, R., Merten, N., Mathiesen, J. M., Martini, L., Kruljac-Letunic, A., Krop, F., et al. (2009). The C-terminal tail of CRTH2 is a key molecular determinant that constrains gαi and downstream signaling cascade activation. J. Biol. Chem. 284, 1324–1336. doi: 10.1074/jbc.M806867200
Selivanova, S. V., Toscano, A., Abate, C., Berardi, F., Müller, A., Krämer, S. D., et al. (2015). Synthesis and pharmacological evaluation of 11C-labeled piperazine derivative as a PET probe for Sigma-2 receptor imaging. Nucl. Med. Biol. 42, 399–405. doi: 10.1016/j.nucmedbio.2014.12.018
Singer, B. F., Bryan, M. A., Popov, P., Robinson, T. E., and Aragona, B. J. (2017). Rapid induction of dopamine sensitization in the nucleus accumbens shell induced by a single injection of cocaine. Behav. Brain Res. 324, 66–70. doi: 10.1016/j.bbr.2017.02.018
Svenningson, P., Nishi, A., Fisone, G., Girault, J.-A., Nairn, A. C., and Greengard, P. (2004). DARPP-32: an integrator of neurotransmission. Annu. Rev. Pharmacol. Toxicol. 44, 269–296. doi: 10.1146/annurev.pharmtox.44.101802.121415
Trifilieff, P., Rives, M. L., Urizar, E., Piskorowski, R. A., Vishwasrao, H. D., Castrillon, J., et al. (2011). Detection of antigen interactions ex vivo by proximity ligation assay: endogenous dopamine D2-adenosine A2A receptor complexes in the striatum. BioTechniques 51, 111–118. doi: 10.2144/00013719
Valjent, E., Corbillé, A.-G., Bertran-Gonzalez, J., Hervé, D., and Girault, J.-A. (2006). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. Proc. Natl. Acad. Sci. U.S.A. 103, 2932–2937. doi: 10.1073/pnas.0511030103
Valjent, E., Pagès, C., Hervé, D., Girault, J.-A., 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. doi: 10.1111/j.1460-9586.2004.03278.x
Wang, L., Ye, J., He, Y., Deuther-Condor, W., Zhang, J., Zhang, X., et al. (2017). 18F-Labeled indole-based analogs as highly selective radioligands for imaging Sigma-2 receptors in the brain. Bioorg. Med. Chem. 25, 3792–3802. doi: 10.1016/j.bmc.2017.05.019
Wise, R. A. (1984). Neural mechanisms of the reinforcing action of cocaine. NIDA Res. Monogr. 50, 15–33.
Zeng, C., Garg, N., and Mach, R. H. (2016). The PGRMC1 protein level correlates with the binding activity of a Sigma-2 fluorescent probe (SW120) in rat brain cells. Mol. Imaging Biol. 18, 172–179. doi: 10.1007/s11307-015-0891-z
Zhang, L., Huang, L., Lu, K., Liu, Y., Tu, G., Zhu, M., et al. (2017). Cocaine-induced synaptic structural modification is differentially regulated by dopamine D1 and D2 receptors-mediated signaling pathways. Addict. Biol. 22, 1842–1855.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Aguinaga, Medrano, Vega-Quiroga, Gysling, Canela, Navarro and Franco. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.