

8-1-2015


Mechanisms of activation of nucleus accumbens neurons by cocaine via sigma-1 receptor-inositol 1,4,5-trisphosphate-transient receptor potential canonical channel pathways.

Jeffrey L. Barr
Temple University School of Medicine

Elena Deliu
Temple University School of Medicine

Gabriela Cristina Brailoiu
Thomas Jefferson University

Pingwei Zhao
Temple University School of Medicine
Follow this and additional works at: <https://jdc.jefferson.edu/pharmacyfp>

 [Digital Commons](#) at the [Pharmacy and Pharmaceutical Sciences Commons](#)
Thomas Jefferson University

[Let us know how access to this document benefits you](#)

See next page for additional authors

Recommended Citation

Barr, Jeffrey L.; Deliu, Elena; Brailoiu, Gabriela Cristina; Zhao, Pingwei; Yan, Guang; Abood, Mary E.; Unterwald, Ellen M.; and Brailoiu, Eugen, "Mechanisms of activation of nucleus accumbens neurons by cocaine via sigma-1 receptor-inositol 1,4,5-trisphosphate-transient receptor potential canonical channel pathways." (2015). *College of Pharmacy Faculty Papers*. Paper 26.
<https://jdc.jefferson.edu/pharmacyfp/26>

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's [Center for Teaching and Learning \(CTL\)](#). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in College of Pharmacy Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Authors

Jeffrey L. Barr, Elena Deliu, Gabriela Cristina Brailoiu, Pingwei Zhao, Guang Yan, Mary E. Abood, Ellen M. Unterwald, and Eugen Brailoiu



Published in final edited form as:

Cell Calcium. 2015 August 1; 58(2): 196–207. doi:10.1016/j.ceca.2015.05.001.

Mechanisms of Activation of Nucleus Accumbens Neurons by Cocaine via Sigma-1 Receptor - Inositol 1,4,5-Trisphosphate - Transient Receptor Potential Canonical Channel Pathways

Jeffrey L. Barr^{a,b}, Elena Deliu^a, G. Cristina Brailoiu^c, Pingwei Zhao^a, Guang Yan^c, Mary E. Abood^{a,d}, Ellen M. Unterwald^{a,b,#}, and Eugen Brailoiu^{a,#}

^aCenter for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

^bDepartment of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, USA

^dDepartment of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140, USA

^cDepartment of Pharmaceutical Sciences, Thomas Jefferson University, Jefferson School of Pharmacy, Philadelphia, PA 19107, USA

Abstract

Cocaine promotes addictive behavior primarily by blocking the dopamine transporter, thus increasing dopamine transmission in the nucleus accumbens (nAcc); however, additional mechanisms are continually emerging. Sigma-1 receptors (σ_1 Rs) are known targets for cocaine, yet the mechanisms underlying σ_1 R-mediated effects of cocaine are incompletely understood. The present study examined direct effects of cocaine on dissociated nAcc neurons expressing phosphatidylinositol-linked D_1 receptors. Endoplasmic reticulum-located σ_1 Rs and inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3 Rs) were targeted using intracellular microinjection. IP_3 microinjection robustly elevated intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. While cocaine alone was devoid of an effect, the IP_3 -induced response was σ_1 R-dependently enhanced by cocaine co-injection. Likewise, cocaine augmented the $[Ca^{2+}]_i$ increase elicited by extracellularly applying an IP_3 -generating molecule (ATP), via σ_1 Rs. The cocaine-induced enhancement of the P_3/ATP -mediated Ca^{2+} elevation occurred at pharmacologically relevant concentrations and was mediated by transient receptor potential canonical channels (TRPC). IP_3 microinjection elicited a slight,

[#]To whom correspondence should be addressed: Eugen Brailoiu, M.D., Center for Substance Abuse Research, Temple University School of Medicine, 3500 N. Broad Street, Room 848, Philadelphia, PA 19140, Tel: 215-707-2791, Fax: 215-707-9890, ebrailou@temple.edu or to: Ellen M Unterwald, Ph.D., Center for Substance Abuse Research, Temple University School of Medicine, 3500 N. Broad Street, Room 860, Philadelphia, PA 19140, Tel: 215-707-1681, Fax: 215-707-7068, ellen.unterwald@temple.edu.

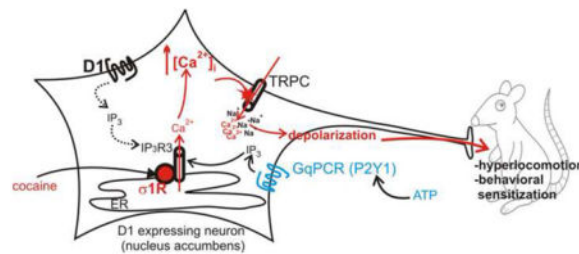
Author contributions: J.L.B., E.D., G.C.B., P.Z., G.Y. and E.B. performed experiments; J.L.B., E.D., G.C.B., P.Z., G.Y., M.E.A., E.M.U. and E.B. analyzed data; E.B. and E.M.U. designed experiments; E.D. and J.L.B. drafted the manuscript; E.B., M.E.A. and E.M.U. finalized the manuscript; all authors read and approved the final version of the manuscript.

The authors declare no competing financial interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

transient depolarization, further converted to a greatly enhanced, prolonged response, by cocaine co-injection. The cocaine-triggered augmentation was σ_1 R-dependent, TRPC-mediated and contingent on $[Ca^{2+}]_i$ elevation. ATP-induced depolarization was similarly enhanced by cocaine. Thus, we identify a novel mechanism by which cocaine promotes activation of D₁-expressing nAcc neurons: enhancement of IP₃R-mediated responses via σ_1 R activation at the endoplasmic reticulum, resulting in augmented Ca^{2+} release and amplified depolarization due to subsequent stimulation of TRPC. *In vivo*, intra-accumbal blockade of σ_1 R or TRPC significantly diminished cocaine-induced hyperlocomotion and locomotor sensitization, endorsing a physio-pathological significance of the pathway identified *in vitro*.

Graphical Abstract



Keywords

sigma receptors; transient receptor potential channels; calcium; endoplasmic reticulum; imaging; nucleus accumbens

1. Introduction

Pharmacotherapy of cocaine addiction is particularly ineffective in that addicts invariably relapse to drug use [1–3]. The study of cocaine action in the brain has unraveled newer mechanisms, of continually emerging complexity. The initial rewarding effects of cocaine are attributed to increased dopamine levels in the nucleus accumbens (nAcc)[¶] achieved by inhibition of the dopamine transporter [4, 5], however, the transition to cocaine dependence likely involves other cellular processes. Further, a multitude of cocaine-induced responses are strictly contingent on D₁ dopamine-receptor activation [6–8], occurring only in D₁-expressing neurons [7], supporting the existence of additional mechanisms.

Cocaine is a known ligand of sigma-1 receptors (σ_1 Rs) [9], which are chaperone proteins residing at the endoplasmic reticulum in a dormant state, but able to relocate to other areas of the cell in response to agonist stimulation, and change their degree of interaction with several other chaperone proteins, favoring activation of various types of receptors and ion channels [10, 11]. Involvement of σ_1 Rs in modulation of dopaminergic transmission and addictive processes has long been recognized [12, 13]. σ_1 Rs are expressed in the nAcc, a key node in the circuit that controls reward-directed behavior [14, 15], and have been proposed as a pharmacologic target in the treatment of cocaine abuse [16, 17].

Data from *in vivo* animal studies point to involvement of σ_1 Rs in cocaine-induced responses. Cocaine self-administration triggers σ_1 Rs-mediated reinforcing effects that are absent in

subjects without that particular experience with cocaine, and are dopamine-independent [18, 19]. Administration of σ_1 R agonists potentiates the reinforcing effects of cocaine [19]. Conversely, σ_1 R antagonists attenuate psychomotor and rewarding effects of cocaine [12, 13]. σ_1 R-blockade inhibits cocaine-induced place conditioning in mice [20, 21].

At the cellular level, cocaine induces an association of σ_1 R and D_1 dopamine receptors, which results in cAMP accumulation and ERK_{1/2} activation in transfected cells and mouse striatal slices [22]. Conversely, cocaine promotes formation of σ_1 R- D_2 dopamine receptor heterooligomers, inhibiting D_2 -mediated signaling [23]. Thus cocaine putatively destabilizes the balance of D_1 and D_2 receptor inputs, via σ_1 R, towards the D_1 containing, pro-reward and motivating pathway [23]. However, a clear mechanism of cocaine-mediated enhancement of D_1 -pathway via σ_1 R remains elusive.

Activation of σ_1 R is associated with prolonged Ca^{2+} efflux from the endoplasmic reticulum (ER) through inositol 1,4,5-trisphosphate (IP₃) receptors (IP₃R) [10, 24]. The present study uses calcium imaging and intracellular microinjection to explore the mechanisms of cocaine-triggered activation of D_1 -expressing neurons, focusing on intracellularly-located σ_1 R.

2. Materials and methods

2.1. Ethical approval

Animal protocols were approved by the Institutional Animal Care and Use Committees from Temple University and Thomas Jefferson University.

2.2. Chemicals

All chemicals were from Sigma Aldrich (St. Louis, MO), unless otherwise mentioned. Cocaine hydrochloride was generously supplied by NIDA; NE-100 hydrochloride was from Santa Cruz Biotechnology (Dallas, TX). In experiments using intracellular microinjection, the reported concentration of chemicals is the calculated final concentration inside the cell. In experiments using ATP and extracellular administration of cocaine, the cells were pretreated with cocaine for 10 minutes, a time sufficient to allow intracellular uptake of cocaine. In experiments using IP₃ and cocaine, there was no pretreatment phase.

2.3. Western blotting

Whole-cell lysates obtained from rat nucleus accumbens and NG108-15 cells (mouse neuroblastoma x rat glioma) were separated on Mini-PROTEAN TGX 4–20% gels (Bio-Rad, Hercules, CA) by SDS-PAGE followed by immunoblotting. Proteins were transferred to an Odyssey nitrocellulose membrane (Li-Cor Biosciences; Lincoln, NE). After blocking with Odyssey blocking buffer, the membranes were incubated overnight with primary antibody against σ_1 R (rabbit polyclonal, 1:100, OriGene Technologies, Rockville, MD), or IP₃R3 (mouse monoclonal, 1:1,000, BD Biosciences, San Jose, CA). An antibody against β -actin (mouse monoclonal, 1:10,000; Sigma Aldrich) was used to confirm equal protein loading. Membranes were washed with Tris-buffered saline-Tween 20 (TBST) and incubated with the secondary antibodies: IRDye 800CW conjugated goat anti-rabbit IgG, and IRDye 680 conjugated goat anti-mouse IgG (1:10,000, 1 h at room temperature).

Membranes were then washed in TBST and scanned using Li-Cor Odyssey Infrared and Odyssey V.3 software.

2.4. Neuronal cell culture

Nucleus accumbens neurons were dissociated from neonatal (1–2 day old) Sprague Dawley rats (Ace Animal Inc., Boyertown, PA) of both sexes as previously described [25]. Newborn rats were decapitated and the brains quickly removed surgically and immersed in ice-cold Hanks balanced salt solution (HBSS) (Mediatech, Herndon, VA). The nucleus accumbens was identified, removed, minced and subjected to enzymatic digestion (papain, 37°C), followed by mechanical trituration in presence of total medium – Neurobasal A (Invitrogen, Carlsbad, CA) containing 1% GlutaMax (Invitrogen), 2% penicillin-streptomycin-amphotericin B solution (Mediatech) and 10% fetal bovine serum. Cells were cultured on round 25 mm glass coverslips coated with poly-L-lysine (Sigma-Aldrich) in six-well plates. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO₂. The mitotic inhibitor cytosine β-arabinofuranoside (1 μM) (Sigma-Aldrich) was added to the culture the third day to inhibit glial cell proliferation. Cells were used after 5 days in culture.

2.5. Calcium imaging

[Ca²⁺]_i was measured as previously described [25]. Cells were incubated with 5 μM fura-2 AM (Invitrogen, Carlsbad, CA) in HBSS at room temperature for 45 min, in the dark, washed three times with dye-free HBSS, and then incubated for another 45 min to allow for complete de-esterification of the dye. Coverslips (25 mm diameter) were subsequently mounted in an open bath chamber (RP-40LP, Warner Instruments, Hamden, CT) on the stage of an inverted microscope Nikon Eclipse TiE (Nikon Inc., Melville, NY). The microscope is equipped with a Perfect Focus System and a Photometrics CoolSnap HQ2 CCD camera (Photometrics, Tucson, AZ). During the experiments, the Perfect Focus System was activated. Fura-2 AM fluorescence (emission = 510 nm), following alternate excitation at 340 and 380 nm, was acquired at a frequency of 0.25 Hz. Images were acquired and analyzed using NIS-Elements AR 3.1 software (Nikon Inc.). After appropriate calibration with ionomycin and CaCl₂, and Ca²⁺ free and EGTA, respectively, the ratio of the fluorescence signals (340/380 nm) was converted to Ca²⁺ concentrations. In Ca²⁺-free experiments, CaCl₂ was omitted.

2.6. Intracellular microinjection

Intracellular microinjections were performed using FemtotipsII, InjectManNI2 and FemtoJet systems (Eppendorf) as reported [25]. Pipettes were back-filled with an intracellular solution containing, in mM: 110 KCl, 10 NaCl and 20 HEPES (pH 7.2) or the compounds to be tested. The injection time was 0.4 s at 60 hPa with a compensation pressure of 20 hPa in order to maintain the microinjected volume to less than 1% of cell volume, as measured by microinjection of a fluorescent compound (Fura-2 free acid). The intracellular concentration of chemicals was determined based on the concentration in the pipette and the volume of injection. The cells to be injected were Z-scanned before injection and the cellular volume automatically calculated by the NIS-Elements AR 3.1 software (Nikon, Inc.).

2.7. Measurement of membrane potential

The relative changes in membrane potential of single neurons were evaluated using bis-(1,3-dibutylbarbituric acid) trimethine oxonol, DiBAC₄(3), a slow response voltage-sensitive dye, as previously described [25]. Upon membrane hyperpolarization, the dye concentrates in the cell membrane, leading to a decrease in fluorescence intensity, while depolarization induces the sequestration of the dye into the cytosol, resulting in an increase of the fluorescence intensity. Cultured accumbens neurons were incubated for 30 min in HBSS containing 0.5 μ M DiBAC₄(3) and the fluorescence monitored at 0.17 Hz, excitation/emission: 480 nm/540 nm. Calibration of DiBAC₄(3) fluorescence following background subtraction was performed using the Na⁺-K⁺ ionophore gramicidin in Na⁺-free physiological solution and various concentrations of K⁺ (to alter membrane potential) and N-methylglucamine (to maintain osmolarity). Under these conditions, the membrane potential was approximately equal to the K⁺ equilibrium potential determined by the Nernst equation. The intracellular K⁺ and Na⁺ concentration were assumed to be 130 mM and 10 mM, respectively.

2.8. Data analysis

Data obtained after *in vitro* experiments are expressed as mean and standard error of mean. One way ANOVA, followed by post-hoc Bonferroni and Tukey tests (Origin 7, OriginLab Corporation, Northampton, MA), were used to assess significant differences between groups; $P < 0.05$ was considered statistically significant.

2.9. In vivo experiments

Male Sprague–Dawley rats, weighing 250–280 g at the time of surgery, were individually housed under standard conditions. Two 26 gauge stainless steel guide cannulas directed bilaterally at the nucleus accumbens (nAcc) (± 0.9 mm lateral, 1.6 mm anterior, and 5.8 mm ventral to bregma) were stereotactically implanted under isofluorane anesthesia. Dummy cannulae that extended 1 mm beyond the tip of the guide cannula were inserted immediately after surgery. Rats were handled and habituated to infusion procedures for 2–3 days before testing began. On test Days 1–5, rats were placed in individual automated activity monitors containing 16 infrared light emitters and sensors mounted on a frame within which a standard plastic animal cage was positioned (45 \times 20 \times 20 cm; AccuScan Instruments, Inc., Columbus, OH, USA). The number of photocell beam breaks was recorded by a computer equipped with Digiscan DMicro software (AccuScan Instruments). Following a 60 min habituation period, bilateral infusions of vehicle (0.5 μ l, artificial cerebrospinal fluid (aCSF)) or BD-1063 (80 μ g/0.5 μ l, ab141323, Abcam, Cambridge, MA) or SKF-96365 (20 μ g/0.5 μ l, S7809, Sigma-Aldrich, St Louis, MO) were made into the nAcc, at a rate of 0.5 μ l/min using a microinfusion pump. The injections cannulae remained in situ for one minute after the infusion. Twenty minutes following infusions, rats were injected with saline (1 ml/kg, intraperitoneal (ip)) or cocaine (15 mg/kg, ip) and behavioral activity monitored for another 60 min. This was repeated once daily for 5 days. Following a 7-day withdrawal period, all rats were then challenged with cocaine (Day 12, 15 mg/kg, ip) in the absence of further intracranial infusions. After testing, brains were removed and fixed in 4% paraformaldehyde for three days. Brains were sliced at 60 μ m on a vibratome through the

nAcc, and stained with cresyl violet to determine the location of the infusion cannula; injection sites are shown in Fig. 9a. Data from rats with both placements within the nAcc were included in the analysis. Behavioral data were analyzed with two-way repeated measures ANOVA. Significant main effects of treatment, day or interactions between treatment and day were further assessed with a Student-Newman-Keuls (SNK) post hoc test for multiple pair wise comparisons at each time point (Sigmaplot 12.5; Systat Software Inc.).

3. Results

3.1. Identification of receptors of interest in cultured nucleus accumbens neurons

We used western blotting to confirm the presence of σ_1 Rs in cultured nAcc neurons (Fig. 1a), which is in agreement with previous reports [14, 15]. σ_1 Rs have been shown to associate with type 3 of IP₃R (IP₃R3) to promote increased Ca²⁺ efflux from the ER [10, 24]. We found that both IP₃R3 and σ_1 Rs are expressed in cultured nAcc neurons (Fig. 1a). NG108 cells were used as a positive control for IP₃R3 and σ_1 Rs [10].

Since several cocaine-mediated responses are strictly dependent on D₁ receptor activation or are restricted to D₁-expressing neurons [6–8], in the present study we used only neurons responding to application of D₁ agonist SKF83959 (10 μ M) with an increase in [Ca²⁺]_i [26, 27] (Fig. 1b); accordingly, these neurons were considered D₁-positive, signaling via Gq-coupled pathways [26]. When incubated with Ca²⁺-free saline, neuronal Ca²⁺ response to application of SKF83959 was reduced from 197 ± 4.6 nM (n = 581 cells in Ca²⁺-containing saline) to 96 ± 3.6 nM (n = 6 cells in Ca²⁺-free medium) and further, largely abolished by presence of IP₃R inhibitors xestospongine C (XeC, 10 μ M, 15 min) and 2-aminoethoxydiphenyl borate (2-APB, 100 μ M, 15 min) – [Ca²⁺]_i = 7 ± 2.1 nM (n = 6, Fig. 1b). This indicates that indeed, in the responsive neurons, SKF83959 promotes Ca²⁺ mobilization from intracellular stores via IP₃Rs, supporting the activity of a Gq-coupled pathway.

3.2. Cocaine enhances IP₃-dependent Ca²⁺ mobilization via σ_1 R activation

In D₁-expressing neurons incubated with Ca²⁺-containing saline, microinjection of cocaine (100 μ M, final concentration inside the cell) did not elicit an increase in [Ca²⁺]_i, the effect being similar to that produced by microinjection of control buffer (Fig. 1c); [Ca²⁺]_i was 28 ± 4.7 nM, and the area under curve of the Ca²⁺ response (A.U.C.) was 33.8 ± 4.4 nM x min for cocaine (n = 6 D₁-positive nAcc neurons), while for control vehicle the effects measured 21 ± 4.2 nM and 36.5 ± 3.7 nM x min (n = 6), respectively (Fig. 1c).

In an additional series of experiments, we tested the effect of intracellular administration cocaine on IP₃-induced Ca²⁺ response in D₁-positive accumbens neurons. To evaluate the effect of cocaine co-injection on the Ca²⁺ mobilization triggered by IP₃, additional experiments were carried out in Ca²⁺-free saline, in order to prevent interference with any Ca²⁺ entry mechanism. At 20 nM, IP₃ microinjection into D₁-positive nAcc neurons produced a Ca²⁺ response of 276 ± 2.8 nM (n = 6 cells), while 10 μ M cocaine co-injected with 20 nM IP₃ triggered a significantly enhanced effect, measuring 364 ± 3.6 nM (n = 6 cells, Fig. 2a). To establish a concentration-response curve, increasing concentrations of IP₃

(1–60 nM) were injected either alone or in combination with 10 μ M cocaine (n = 5 to 6 cells for each concentration tested). The two concentration-response curves are presented in Fig. 2b; cocaine significantly shifted the IP₃ concentration-response curve to the left, diminishing the EC₅₀ for IP₃ from 22.4 nM (when administered alone) to 17.8 nM (when co-administered with cocaine).

Blocking σ_1 Rs with either the selective and prototypical σ_1 R antagonist BD-1063 [28] (10 μ M, 20 min) or with NE-100 (3 μ M, 20 min), another selective σ_1 R inhibitor [29, 30], was sufficient to prevent cocaine-induced augmentation of IP₃-mediated Ca²⁺ mobilization:

[Ca²⁺]_i were 291 \pm 3.3 nM (BD-1063 pretreatment, n = 6 cells) and 285 \pm 3.2 nM (NE-100 pretreatment, n = 6) compared with 364 \pm 3.6 nM (10 μ M cocaine co-injected with 20 nM IP₃, no antagonists, n = 6) and 276 \pm 2.8 nM (20 nM IP₃ alone, n = 6 cells, Fig. 2a, c).

Next, we evaluated the effect of cocaine microinjection on the IP₃-induced Ca²⁺ mobilization in Ca²⁺-containing saline-incubated D₁-positive neurons. IP₃ (20 nM) microinjection alone robustly elevated [Ca²⁺]_i by 332 \pm 4.8 nM (A.U.C. of 111 \pm 4.4 nM x min, n = 6 cells), while in the presence of 10 μ M co-injected cocaine, the effect was greatly enhanced, measuring 576 \pm 5.3 nM in amplitude (A.U.C. of 234 \pm 6.1 nM x min, n = 6) (Fig. 3a, b). Incubation of neurons with BD-1063 (10 μ M, 20 min) reduced the Ca²⁺ response to co-injected cocaine and IP₃ to that of IP₃ alone ([Ca²⁺]_i was 337 \pm 5.8 nM, A.U.C. was 104 \pm 3.3 nM x min, n = 6, Fig. 3a, b), indicating that the cocaine-triggered augmentation was σ_1 R-mediated. This conclusion is supported by the inability of BD-1063 to reduce the effect IP₃ microinjection alone ([Ca²⁺]_i was 342 \pm 5.2 nM, A.U.C. was 114 \pm 4.8 nM x min, Fig. 3a, b). To further strengthen our findings, we evaluated whether the σ_1 R antagonist NE-100 would block the cocaine-mediated enhancement of IP₃-induced Ca²⁺ response: indeed, similar to the effects seen in presence of extracellular Ca²⁺, 20 min pretreatment of neurons with 3 μ M NE-100 resulted in a significant reduction of the Ca²⁺ increase promoted by IP₃ and cocaine co-injection, the response measuring 341 \pm 5.2 nM in amplitude and having an A.U.C. of 108 \pm 4.8 nM x min (Fig. 3a, b). Noteworthy, σ_1 R blockade by either BD-1063 or NE-100 had largely identical diminishing effect on the response triggered by combined cocaine and IP₃ administration, both in the presence and in the absence of extracellular Ca²⁺.

In presence of the fast Ca²⁺ chelator BAPTA-AM (200 μ M, 30 min incubation), combined intracellular administration of cocaine and IP₃ produced a small and insignificant response, measuring 49 \pm 4.1 nM in amplitude and with an A.U.C. of 14 \pm 3.3 nM x min (n = 6, Fig. 3a, b). In presence of SKF96365 (2 μ M), that blocks receptor- and store-operated Ca²⁺ entry via transient receptor potential canonical (TRPC) channels [31, 32], the cocaine-induced potentiation was abolished ([Ca²⁺]_i was 268 \pm 6.4 nM, A.U.C. of 76 \pm 4.6 nM x min, n = 6, Fig. 3a, b). Fig. 3c depicts representative examples of changes in 340 nm/380 nm Fura-2 fluorescence ratio of nAcc neurons in presence of D₁ agonist SKF83959, followed (after washing of SKF83959) by intracellular microinjection of IP₃ alone or IP₃ and cocaine, in absence and presence of the indicated antagonists.

Next, we tested whether cocaine might amplify Ca²⁺ responses induced by another intracellular Ca²⁺ release mediator, cyclic ADP ribose (cADPR), which acts on ryanodine

receptors [33]. Because cADPR has also been involved in the activation of TRPM2 (also known as TRPC7 or LTRPC2) [34, 35], to avoid any TRPC-mediated effect, experiments were performed in absence of extracellular Ca^{2+} . In cells incubated with Ca^{2+} -free saline, cADPR (20 μM) injection elevated $[\text{Ca}^{2+}]_i$ by 174 ± 2.8 nM (A.U.C. of 52 ± 1.3 nM x min, $n = 6$ cells Fig. 4a, b). Cocaine (10 μM) and cADPR (20 μM) co-injection induced an similar response to that of cADPR alone, measuring 171 ± 2.6 nM in amplitude (A.U.C. of 54 ± 1.1 nM x min, $n = 6$, Fig. 4a, b), while microinjection of a higher concentration of cADPR (50 μM) induced a proportionally higher response, of 629 ± 3.7 nM (A.U.C. of 326 ± 2.8 nM x min, $n = 6$ cells, Fig. 4a, b), indicating that 20 μM cADPR produced a submaximal effect. Thus, cocaine does not enhance cADPR-mediated Ca^{2+} signaling in nAcc neurons.

Since diacylglycerol (DAG) has been reported to promote TRPC activation downstream of phospholipase C [36], we tested the effect of the membrane-permeable DAG analogue 1-oleoyl-2-acetyl-sn-glycerol (OAG) on D_1 -expressing nAcc neurons. Application of OAG (75 μM) in cells incubated with Ca^{2+} -free saline elicited no effect (Fig. 4c), while addition of Ca^{2+} to the extracellular medium unmasked a response measuring 251 ± 7.8 nM (A.U.C. of 1183 ± 9.3 nM x min, $n = 12$ neurons, Fig. 4c, d). A likewise effect was produced by 75 μM OAG in cells treated with 10 μM cocaine: no response in absence of extracellular Ca^{2+} and an $[\text{Ca}^{2+}]_i$ elevation by 258 ± 6.7 nM (A.U.C. of 1164 ± 9.7 nM x min, $n = 12$, Fig. 4c, d) upon changing to Ca^{2+} -containing saline. Since absence of a cocaine-enhancing effect may occur as a consequence of the employment of a concentration of OAG eliciting a maximal response, we tested the effect of 100 μM OAG and noted an increase in $[\text{Ca}^{2+}]_i$ by 383 ± 11.8 nM upon Ca^{2+} addition (A.U.C. of 1847 ± 10.2 nM x min, $n = 12$, Fig. 4c, d), significantly higher than that produced by 75 μM OAG.

In D_1 -positive nAcc neurons, the TRPC antagonist SKF96365 (2 μM) did not block the Ca^{2+} elevation in response to KCl (30 mM) (Fig. 5), which produces depolarization-induced activation of voltage-gated Ca^{2+} channels. Thus, as we have previously reported [25], at 2 μM SKF96365 has no antagonistic effect on voltage-gated Ca^{2+} channels.

3.3. Cocaine produces $\sigma_1\text{R}$ -mediated potentiation of the Ca^{2+} response triggered by ATP

Next, we examined the effect of cocaine on ATP, an IP_3 -generating molecule, in D_1 -expressing accumbens neurons. To confirm that ATP mobilizes IP_3 -sensitive Ca^{2+} stores in nAcc neurons, we first examined the effect of extracellular application of ATP (20 μM) on $[\text{Ca}^{2+}]_i$ in Ca^{2+} -free saline, in the absence and presence of inhibitors of endoplasmic reticulum and lysosomal Ca^{2+} release channels. In the absence of extracellular Ca^{2+} , ATP (20 μM) elevated $[\text{Ca}^{2+}]_i$ of nAcc neurons by 278 ± 3.6 nM (A.U.C. of 78.8 ± 3.1 nM x min, $n = 9$ cells, Fig. 6a, b). Blockade of IP_3Rs with XeC (10 μM , 15 min) and 2-APB (100 μM , 15 min), but not inhibition of ryanodine receptors with ryanodine (Ry, 10 μM , 1h) or of lysosomal NAADP-sensitive two pore channels with Ned-19 (5 μM , 15 min) [37], abolished the Ca^{2+} response of nAcc neurons to ATP, indicating that it was IP_3R -mediated (Fig. 6a). In the presence of these blockers, ATP increased $[\text{Ca}^{2+}]_i$ by 23 ± 2.7 nM (XeC + 2-APB; A.U.C. of 5.6 ± 1.8 nM x min, $n = 9$ cells), by 269 ± 3.1 nM (Ry; A.U.C. of 74.7 ± 3.8 nM x min, $n = 9$) and by 274 ± 4.9 nM (Ned-19; A.U.C. of 77.3 ± 3.7 nM x min, $n = 9$), respectively (Fig. 6b).

In Ca^{2+} -containing saline, application of ATP (20 μM) alone elevated $[\text{Ca}^{2+}]_i$ of D_1 -positive neurons by 377 ± 4.8 nM (A.U.C. of 108 ± 4.4 nM x min, $n = 29$ cells, Fig. 6c, d). Combined administration of cocaine (10 μM) and ATP (20 μM) produced a greatly potentiated increase in $[\text{Ca}^{2+}]_i$, measuring 613 ± 8.6 nM in amplitude and with an A.U.C. of 247 ± 4.3 nM x min ($n = 47$ cells, Fig. 6c, d). The cocaine-induced enhancement was completely abrogated by pretreatment of cells with σ_1 R antagonist BD-1063 (10 μM , 20 min) or with the TRPC blocker SKF96365 (2 μM , 20 min), when the Ca^{2+} response was similar to that promoted by ATP alone; $[\text{Ca}^{2+}]_i$ was 364 ± 6.1 nM (A.U.C. of 103 ± 4.6 nM x min, $n = 31$) in the case of BD-1063 and 272 ± 5.7 nM (A.U.C. of 79 ± 5.2 nM x min, $n = 28$) in the case of SKF96365 (Fig. 6c, d). In presence of BAPTA-AM (200 μM , 30 min), co-administration of cocaine and ATP no longer elicited a significant increase in $[\text{Ca}^{2+}]_i$ (amplitude of 23 ± 3.5 nM, A.U.C. of 15 ± 2.3 nM x min, $n = 42$, Fig. 6c, d).

3.4. Intracellular microinjection of cocaine amplifies IP_3 -induced depolarization via σ_1 Rs

The mean resting potential of dissociated D_1 -positive nAcc neurons was -71.6 ± 0.02 mV ($n = 258$ cells depolarizing in response to SKF83959 in the preliminary screen test).

Microinjection of cocaine or control vehicle had no effect on neuronal membrane potential;

V_m were -1.2 ± 0.34 mV ($n = 6$) and -0.9 ± 0.47 mV ($n = 6$), respectively, (Fig. 7a, b).

Intracellular administration of IP_3 (20 nM) produced a slight and rather transient depolarization of 5.12 ± 0.42 mV, which in the presence of co-injected cocaine (10 μM) was converted to a greatly enhanced and more prolonged response, measuring 9.77 ± 0.53 mV in amplitude (Fig. 7c, d). The cocaine-dependent component of the effect was abolished upon blocking σ_1 Rs with BD-1063 (10 μM , 20 min pretreatment), when the combined administration of IP_3 and cocaine depolarized neuronal membrane potential by 5.53 ± 0.39 mV ($n = 6$), similar to IP_3 injection alone (Fig. 7c, d). In presence of the Ca^{2+} chelator BAPTA-AM (200 μM , 30 min) or of TRPC inhibitor SKF96365 (2 μM , 20 min), the IP_3 and cocaine-triggered depolarization were lost, the changes in resting membrane potential measuring 0.79 ± 0.62 mV ($n = 6$) and 1.34 ± 0.47 mV ($n = 6$), respectively (Fig. 7c, d).

3.5. Cocaine enhances ATP-elicited depolarization of D_1 -positive nAcc neurons

Cocaine produced a likewise augmentation of the amplitude and duration of the depolarization promoted by the IP_3 -generating molecule ATP (Fig. 8a). ATP (20 μM) depolarized D_1 -expressing nAcc neurons by 6.71 ± 0.39 mV ($n = 36$) in absence and by 11.63 ± 0.54 ($n = 52$ cells) in presence of co-applied cocaine (10 μM) (Fig. 8b). Pretreatment of cells with σ_1 R blocker BD-1063 (10 μM , 20 min) virtually eliminated the cocaine-mediated amplification, as neurons depolarized only by 7.38 ± 0.47 mV ($n = 46$) when ATP and cocaine were co-applied in this condition (Fig. 8a, b). The depolarization triggered by combined administration of cocaine and ATP measured 5.68 ± 0.53 mV ($n = 39$) when neurons were preincubated with the fast Ca^{2+} chelator BAPTA-AM (200 μM , 30 min) and 5.92 ± 0.41 mV ($n = 43$) upon pretreatment with TRPC blocker SKF96365 (2 μM , 20 min) (Fig. 8a, b). These results support a contribution of the $[\text{Ca}^{2+}]_i$ elevation and of cation entry via TRPC in the mechanism of cocaine-induced enhancement of ATP effects in D_1 -positive nAcc neurons.

3.6. Blockade of σ_1 Rs or TRPC in the nAcc reverses cocaine-induced hyperlocomotion and sensitization in vivo

The role of σ_1 Rs and TRPC in the nAcc in cocaine-induced hyperactivity was investigated in the rat. To this end, the ability of the selective σ_1 R antagonist BD-1063 (80 μ g/0.5 μ l, intra-nAcc), and the TRPC blocker SKF96365 (20 μ g/0.5 μ l, intra-nAcc), to attenuate acute cocaine (15 mg/kg ip)-induced hyperlocomotion was tested. Two-way ANOVA with repeated measures over days revealed a statistically significant main effect of day ($F_{5,202} = 22.821$, $p < 0.001$), a significant treatment main effect ($F_{5,202} = 13.994$, $p < 0.001$) and no treatment \times day interaction ($F_{25,202} = 1.296$, $p = 0.175$). Post test analysis revealed that administration of cocaine following aCSF infusion produced a significant increase in ambulatory activity on Days 2–5 compared with aCSF + vehicle administration (Fig. 8b; aC vs aV; $p < 0.05$). Pretreatment with BD-1063 or SKF96365 into the accumbens significantly inhibited cocaine-induced locomotion on Days 2–5 (aC vs BC or SC, $p < 0.05$). Infusion of BD-1063 or SKF96365 alone (prior to a saline injection) did not alter locomotion when compared to the levels of activity after aCSF infusion and saline injection (Fig. 8b, BV or SV vs aV; $p > 0.05$). On day 5, activity of the SKF96365 + cocaine group was different from the three vehicle control groups (SC vs aV/BV/SV; post hoc $p < 0.05$), but significantly lower than the cocaine alone group (SC vs aV; $p < 0.05$).

Locomotor sensitization occurs as the result of repeated cocaine administration. The ability of the BD-1063 and SKF96365 to block the development of locomotor sensitization to repeated cocaine was investigated. Seven days following the five daily treatments described above, all rats were challenged with cocaine (15 mg/kg ip) on Day 12 without further intracranial infusions and activity recorded. Rats receiving daily intra-accumbens aCSF plus cocaine for 5 days showed significantly greater ambulatory response to the cocaine challenge on Day 12 than did rats receiving daily intra-accumbens aCSF plus saline, demonstrating locomotor sensitization (Fig. 9b; aC vs aV, $p < 0.05$). Activity following the cocaine challenge of the groups receiving daily intra-accumbens BD-1063 or SKF96365 plus cocaine was significantly lower than those receiving aCSF plus cocaine (BC/SC vs aC, $p < 0.05$). These data demonstrate that cocaine-induced hyperactivity and sensitization can be blocked by pretreatment with the σ_1 R antagonist BD-1063 or TRPC blocker SFK96365.

4. Discussion

Emerging evidence points to IP₃ involvement both in σ_1 R-mediated signaling and in cocaine-promoted responses. σ_1 R activation potentiates not only IP₃R-mediated Ca²⁺ release from the ER [24], but also IP₃ formation [38, 39]. IP₃R blockade at central levels inhibits cocaine-induced place preference in mice [40]. Cocaine binds to σ_1 Rs with a 2 μ M affinity [9]. *In vivo* studies have shown that pharmacologically relevant doses of cocaine produce striatal levels of the drug in a low μ M range [41, 42]. For instance, it has been reported that after systemic administration to mice of a 10 mg/kg dose of cocaine, a peak value for cocaine of 2.6 μ g/g (~7.6 – 8.5 μ M) at 5 min was seen in the brain, whereas after a 25 mg/kg the peak value was 6.7 μ g/g (~19.7 – 22.1 μ M) [41]; moreover, the cocaine concentrations in the brain were always higher than those in the plasma, with an average brain/plasma ratio of 7 [41].

We report here that, although ineffective by itself at concentrations up to 100 μM , at 10 μM cocaine strongly enhances IP_3 -induced Ca^{2+} responses in D_1 -expressing nAcc neurons by activating intracellularly-located $\sigma_1\text{Rs}$, significantly shifting the IP_3 concentration-response curve to the left. Our finding is supported by previous indications that in NG108 cells, cocaine promotes $\sigma_1\text{R}$ -dependent increase in $\text{IP}_3\text{R3}$ sensitivity for IP_3 , further translated into augmented Ca^{2+} efflux from the ER in the presence of IP_3 -generating mediators such as bradykinin [10, 43]. Accordingly, we found that the Ca^{2+} response induced by ATP (which clearly mobilized only IP_3 -sensitive intracellular Ca^{2+} stores in our paradigm) was similarly enhanced by cocaine via $\sigma_1\text{Rs}$. Conversely, cocaine had no effect on the ryanodine receptor-mediated Ca^{2+} release.

We note two potential implications of our findings. On the one hand, cocaine is a dopamine transporter blocker, producing indirect activation of phospholipase C β -coupled D_1 -like receptors, which results in IP_3 accumulation in the striatum [44]. Thus, one putative mechanism of cocaine-induced activation of D_1 -positive neurons *in vivo* would include two steps: a dopamine-dependent increase in IP_3 levels, likely associated with an increase in $[\text{Ca}^{2+}]_i$, followed by a dopamine-independent, cocaine-directed activation of $\sigma_1\text{Rs}$, resulting in potentiation of the initial Ca^{2+} response.

On the other hand, increased levels of ATP synthase β -chain have been found in the nAcc of cocaine-overdose victims [45], suggesting that cocaine may elicit local ATP elevation in this brain region. $\sigma_1\text{Rs}$ promote ATP production via increased mitochondrial Ca^{2+} uptake [46] and potentiate ATP-triggered Ca^{2+} mobilization [47]. ATP induces IP_3 -mediated Ca^{2+} release by activating Gq-coupled $\text{P2Y}(1)$ receptors, which are expressed by neurons in the nAcc [48]. In view of our present findings, once ATP levels in the nAcc increase, cocaine is expected to directly activate D_1 -positive neurons, by $\sigma_1\text{R}$ -dependently enhancing ATP effects.

Another important result of our study is that cocaine-triggered enhancement of IP_3 - or ATP-mediated Ca^{2+} elevation was proportionally translated into a $\sigma_1\text{R}$ -dependent augmentation of IP_3 - or ATP-induced depolarization of D_1 -expressing nAcc neurons. Experiments using the fast Ca^{2+} chelator BAPTA-AM clearly indicate contingency of the depolarization on $[\text{Ca}^{2+}]_i$ increase.

Changes in resting membrane potential determine the ease with which excitatory synaptic inputs bring the membrane potential closer to the threshold for action potential firing. *In vivo*, the membrane potential of D_1 -positive medium spiny neurons oscillates between “down-states” characterized by highly negative values (ranging from -75 to -85 mV) and “up-states” consisting in periodic plateau depolarizations ($V_m \sim -55$ mV) triggered by convergent, temporally coherent excitatory synaptic inputs [49, 50]. Accordingly, we found that the resting membrane potential of dissociated D_1 -positive neurons was ~ -72 mV. Given that dopamine depolarizes D_1 -expressing neurons and that under conditions of cocaine administration synaptic dopamine levels are increased, we propose that cocaine-induced $\sigma_1\text{R}$ activation favors the D_1 pathway in the nAcc. Indeed, we found that cocaine-mediated locomotor hyperactivity and sensitization is significantly attenuated by prior administration of a $\sigma_1\text{R}$ antagonist into the rat nAcc.

IP₃-dependent Ca²⁺ signaling has been shown to be involved in the activation of TRPC [25, 51]. TRPC subtypes 4 and 5 are expressed in the rodent nAcc at low and moderate levels, respectively [52], and have been implicated in cocaine-triggered motivation/reward-directed behaviors [53, 54]. Rats lacking a functional *trpc4* gene showed reduced cocaine self-administration [54]; mice with selective *trpc5* knock-down in the forebrain exhibited increased cocaine self-administration on the first day of testing, although were similar to wild-type mice during the maintenance phase of self-administration [53]. We report here that TRPC are critical for cocaine-triggered amplification of IP₃/ATP-dependent Ca²⁺ responses and membrane potential changes in D₁-expressing nAcc neurons. Importantly, cocaine does not enhance OAG-mediated Ca²⁺ signaling, indication that IP₃ is a critical molecule in the pathway linking cocaine and TRPC. Moreover, the TRPC-involving route in the nAcc mediates cocaine-induced hyperlocomotion and locomotor sensitization, as indicated by our experiments using intra-accumbal delivery of a TRPC blocker.

Thus, we propose a new mechanism by which cocaine stimulates or maintains D₁-positive neurons in active state: σ₁R activation at the ER, resulting in dissociation from inhibitory proteins [10, 11, 43] and consequent increases in IP₃R3 sensitivity for IP₃ [10, 43]. This in turn correlates with increased Ca²⁺ efflux from the ER, which promotes TRPC opening [25, 51] and cation entry, resulting in further [Ca²⁺]_i increase and membrane depolarization, converging to locomotor hyperactivity and sensitization (Fig. 10).

Previous studies examining cocaine-induced σ₁R activation noted a potential cocaine-mediated translocation of σ₁R from the ER to the plasma membrane of cells in striatal slices, where σ₁Rs interact with a D₁-D₁ homomer [22] or a heteromer of D₁ and H₃ histamine receptor [55], thus facilitating D₁-mediated effects. Another σ₁R-dependent effect of cocaine involves formation of higher order oligomers between σ₁R and D₂ receptors in mouse striatal slices and inhibition of signaling via D₂ [23]. These plasma membrane-initiated mechanisms were clearly demonstrated in transfected cells treated with 15–30 μM cocaine, while their potential occurrence in brain striatal slices was apparent at higher cocaine concentrations (150 μM) meant to allow diffusion into the tissue [22, 23, 55]. Another study identified a cocaine-induced association of σ₁R and K⁺ channels at the plasmalemma of nAcc shell neurons with implications for cocaine-promoted changes in neuronal excitability and associated behavioral sensitization [56]. Our study is the first indication of σ₁R-directed effects of cocaine occurring upon their intracellular activation in nAcc neurons, and not upon translocation at the plasma membrane. Moreover, we identify a previously unknown mechanism of cocaine action in D₁-expressing nAcc neurons, involving TRPC activation, providing a putative explanation of the *in vivo* results here shown, as well as those of others [54].

Acknowledgments

This work was supported by the NIH grants HL090804 (to EB), DA035926 (to MEA), P30DA013429 (to EMU).

Abbreviations The abbreviations used are

aCSF artificial cerebrospinal fluid

ANOVA	analysis of variance
A.U.C	area under curve
BAPTA	1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis
[Ca²⁺]_i	intracellular Ca ²⁺ concentration
DiBAC₄(3)	bis-(1,3-dibutylbarbituric acid) trimethine oxonol
ER	endoplasmic reticulum
HBSS	Hank's balanced salt solution
IP₃	inositol 1,4,5-trisphosphate
IP₃R	IP ₃ receptor
nAcc	nucleus accumbens
σ₁R	sigma-1 receptor
TRPC	transient receptor potential canonical channels

References

1. Carroll FI, Howell LL, Kuhar MJ. Pharmacotherapies for treatment of cocaine abuse: preclinical aspects. *J Med Chem.* 1999; 42:2721–36. [PubMed: 10425082]
2. Gorelick DA, Gardner EL, Xi ZX. Agents in development for the management of cocaine abuse. *Drugs.* 2004; 64:1547–73. [PubMed: 15233592]
3. Rush CR, Stoops WW. Agonist replacement therapy for cocaine dependence: a translational review. *Future Med Chem.* 2012; 4:245–65. [PubMed: 22300101]
4. Chen R, Tilley MR, Wei H, Zhou F, Zhou FM, Ching S, Quan N, Stephens RL, Hill ER, Nottoli T, Han DD, Gu HH. Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proc Natl Acad Sci U S A.* 2006; 103:9333–8. [PubMed: 16754872]
5. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature.* 1996; 379:606–12. [PubMed: 8628395]
6. Anderson SM, Pierce RC. Cocaine-induced alterations in dopamine receptor signaling: implications for reinforcement and reinstatement. *Pharmacol Ther.* 2005; 106:389–403. [PubMed: 15922019]
7. Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamalas M, Herve D, Valjent E, Girault JA. Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J Neurosci.* 2008; 28:5671–85. [PubMed: 18509028]
8. Xu M, Hu XT, Cooper DC, Moratalla R, Graybiel AM, White FJ, Tonegawa S. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. *Cell.* 1994; 79:945–55. [PubMed: 8001143]
9. Sharkey J, Glen KA, Wolfe S, Kuhar MJ. Cocaine binding at sigma receptors. *Eur J Pharmacol.* 1988; 149:171–4. [PubMed: 2840298]
10. Hayashi T, Su TP. Regulating ankyrin dynamics: Roles of sigma-1 receptors. *Proc Natl Acad Sci U S A.* 2001; 98:491–6. [PubMed: 11149946]
11. Su TP, Hayashi T, Maurice T, Buch S, Ruoho AE. The sigma-1 receptor chaperone as an inter-organellar signaling modulator. *Trends Pharmacol Sci.* 2010; 31:557–66. [PubMed: 20869780]
12. Katz JL, Su TP, Hiranita T, Hayashi T, Tanda G, Kopajtic T, Tsai SY. A Role for Sigma Receptors in Stimulant Self Administration and Addiction. *Pharmaceuticals (Basel).* 2011; 4:880–914. [PubMed: 21904468]

13. Maurice T, Su TP. The pharmacology of sigma-1 receptors. *Pharmacol Ther.* 2009; 124:195–206. [PubMed: 19619582]
14. Alonso G, Phan V, Guillemain I, Saunier M, Legrand A, Anoa M, Maurice T. Immunocytochemical localization of the sigma(1) receptor in the adult rat central nervous system. *Neuroscience.* 2000; 97:155–70. [PubMed: 10771347]
15. Gundlach AL, Largent BL, Snyder SH. Autoradiographic localization of sigma receptor binding sites in guinea pig and rat central nervous system with (+)3H-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine. *J Neurosci.* 1986; 6:1757–70. [PubMed: 3012017]
16. Matsumoto RR. Targeting sigma receptors: novel medication development for drug abuse and addiction. *Expert Rev Clin Pharmacol.* 2009; 2:351–8. [PubMed: 22112179]
17. Matsumoto RR, Liu Y, Lerner M, Howard EW, Brackett DJ. Sigma receptors: potential medications development target for anti-cocaine agents. *Eur J Pharmacol.* 2003; 469:1–12. [PubMed: 12782179]
18. Hiranita T, Mereu M, Soto PL, Tanda G, Katz JL. Self-administration of cocaine induces dopamine-independent self-administration of sigma agonists. *Neuropsychopharmacology.* 2013; 38:605–15. [PubMed: 23187725]
19. Hiranita T, Soto PL, Tanda G, Katz JL. Reinforcing effects of sigma-receptor agonists in rats trained to self-administer cocaine. *J Pharmacol Exp Ther.* 2010; 332:515–24. [PubMed: 19892920]
20. Romieu P, Martin-Fardon R, Maurice T. Involvement of the sigma1 receptor in the cocaine-induced conditioned place preference. *Neuroreport.* 2000; 11:2885–8. [PubMed: 11006959]
21. Romieu P, Phan VL, Martin-Fardon R, Maurice T. Involvement of the sigma(1) receptor in cocaine-induced conditioned place preference: possible dependence on dopamine uptake blockade. *Neuropsychopharmacology.* 2002; 26:444–55. [PubMed: 11927169]
22. Navarro G, Moreno E, Aymerich M, Marcellino D, McCormick PJ, Mallol J, Cortes A, Casado V, Canela EI, Ortiz J, Fuxe K, Lluís C, Ferre S, Franco R. Direct involvement of sigma-1 receptors in the dopamine D1 receptor-mediated effects of cocaine. *Proc Natl Acad Sci U S A.* 2010; 107:18676–81. [PubMed: 20956312]
23. Navarro G, Moreno E, Bonaventura J, Brugarolas M, Farre D, Aguinaga D, Mallol J, Cortes A, Casado V, Lluís C, Ferre S, Franco R, Canela E, McCormick PJ. Cocaine inhibits dopamine D2 receptor signaling via sigma-1-D2 receptor heteromers. *PLoS One.* 2013; 8:e61245. [PubMed: 23637801]
24. Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell.* 2007; 131:596–610. [PubMed: 17981125]
25. Deliu E, Brailoiu GC, Eguchi S, Hoffman NE, Rabinowitz JE, Tilley DG, Madesh M, Koch WJ, Brailoiu E. Direct evidence of intracrine angiotensin II signaling in neurons. *Am J Physiol Cell Physiol.* 2014; 306:C736–44. [PubMed: 24401846]
26. Chun LS, Free RB, Doyle TB, Huang XP, Rankin ML, Sibley DR. D1-D2 dopamine receptor synergy promotes calcium signaling via multiple mechanisms. *Mol Pharmacol.* 2013; 84:190–200. [PubMed: 23680635]
27. Jin LQ, Goswami S, Cai G, Zhen X, Friedman E. SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. *J Neurochem.* 2003; 85:378–86. [PubMed: 12675914]
28. Matsumoto RR, Bowen WD, Tom MA, Vo VN, Truong DD, De Costa BR. Characterization of two novel sigma receptor ligands: antidystonic effects in rats suggest sigma receptor antagonism. *Eur J Pharmacol.* 1995; 280:301–10. [PubMed: 8566098]
29. Berardi F, Ferorelli S, Colabufo NA, Leopoldo M, Perrone R, Tortorella V. A multireceptorial binding reinvestigation on an extended class of sigma ligands: N-[omega-(indan-1-yl and tetralin-1-yl)alkyl] derivatives of 3,3-dimethylpiperidine reveal high affinities towards sigma1 and EBP sites. *Bioorg Med Chem.* 2001; 9:1325–35. [PubMed: 11377189]
30. Chaki S, Okuyama S, Ogawa S, Tanaka M, Muramatsu M, Nakazato A, Tomisawa K. Solubilization and characterization of binding sites for [3H]NE-100, a novel and potent sigma 1 ligand, from guinea pig brain. *Life Sci.* 1996; 59:1331–40. [PubMed: 8876662]

31. Clapham DE, Julius D, Montell C, Schultz G. International Union of Pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. *Pharmacol Rev.* 2005; 57:427–50. [PubMed: 16382100]
32. Zhu X, Jiang M, Birnbaumer L. Receptor-activated Ca²⁺ influx via human Trp3 stably expressed in human embryonic kidney (HEK)293 cells. Evidence for a non-capacitative Ca²⁺ entry. *J Biol Chem.* 1998; 273:133–42. [PubMed: 9417057]
33. Guse AH. Calcium mobilizing second messengers derived from NAD. *Biochim Biophys Acta.* 2014
34. Kolisek M, Beck A, Fleig A, Penner R. Cyclic ADP-ribose and hydrogen peroxide synergize with ADP-ribose in the activation of TRPM2 channels. *Mol Cell.* 2005; 18:61–9. [PubMed: 15808509]
35. Togashi K, Hara Y, Tominaga T, Higashi T, Konishi Y, Mori Y, Tominaga M. TRPM2 activation by cyclic ADP-ribose at body temperature is involved in insulin secretion. *EMBO J.* 2006; 25:1804–15. [PubMed: 16601673]
36. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem.* 2007; 76:387–417. [PubMed: 17579562]
37. Naylor E, Arredouani A, Vasudevan SR, Lewis AM, Parkesh R, Mizote A, Rosen D, Thomas JM, Izumi M, Ganesan A, Galione A, Churchill GC. Identification of a chemical probe for NAADP by virtual screening. *Nat Chem Biol.* 2009; 5:220–6. [PubMed: 19234453]
38. Novakova M, Ela C, Bowen WD, Hasin Y, Eilam Y. Highly selective sigma receptor ligands elevate inositol 1,4,5-trisphosphate production in rat cardiac myocytes. *Eur J Pharmacol.* 1998; 353:315–27. [PubMed: 9726662]
39. Tsai SY, Hayashi T, Mori T, Su TP. Sigma-1 receptor chaperones and diseases. *Cent Nerv Syst Agents Med Chem.* 2009; 9:184–9. [PubMed: 20021352]
40. Kurokawa K, Mizuno K, Shibasaki M, Ohkuma S. Regulation of type 1 inositol 1,4,5-trisphosphate receptor by dopamine receptors in cocaine-induced place conditioning. *Synapse.* 2012; 66:180–6. [PubMed: 22033896]
41. Benuck M, Lajtha A, Reith ME. Pharmacokinetics of systemically administered cocaine and locomotor stimulation in mice. *J Pharmacol Exp Ther.* 1987; 243:144–9. [PubMed: 3668848]
42. Pettit HO, Pan HT, Parsons LH, Justice JB Jr. Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. *J Neurochem.* 1990; 55:798–804. [PubMed: 2384752]
43. Su TP, Hayashi T. Cocaine affects the dynamics of cytoskeletal proteins via sigma(1) receptors. *Trends Pharmacol Sci.* 2001; 22:456–8. [PubMed: 11543872]
44. Medvedev IO, Ramsey AJ, Masoud ST, Bermejo MK, Urs N, Sotnikova TD, Beaulieu JM, Gainetdinov RR, Salahpour A. D1 dopamine receptor coupling to PLCbeta regulates forward locomotion in mice. *J Neurosci.* 2013; 33:18125–33. [PubMed: 24227722]
45. Tannu N, Mash DC, Hemby SE. Cytosolic proteomic alterations in the nucleus accumbens of cocaine overdose victims. *Mol Psychiatry.* 2007; 12:55–73. [PubMed: 17075605]
46. Shioda N, Ishikawa K, Tagashira H, Ishizuka T, Yawo H, Fukunaga K. Expression of a truncated form of the endoplasmic reticulum chaperone protein, sigma1 receptor, promotes mitochondrial energy depletion and apoptosis. *J Biol Chem.* 2012; 287:23318–31. [PubMed: 22619170]
47. Wu Z, Bowen WD. Role of sigma-1 receptor C-terminal segment in inositol 1,4,5-trisphosphate receptor activation: constitutive enhancement of calcium signaling in MCF-7 tumor cells. *J Biol Chem.* 2008; 283:28198–215. [PubMed: 18539593]
48. Franke H, Kittner H, Grosche J, Illes P. Enhanced P2Y1 receptor expression in the brain after sensitisation with d-amphetamine. *Psychopharmacology (Berl).* 2003; 167:187–94. [PubMed: 12652343]
49. Wilson CJ. The generation of natural firing patterns in neostriatal neurons. *Prog Brain Res.* 1993; 99:277–97. [PubMed: 8108553]
50. Wilson CJ, Kawaguchi Y. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J Neurosci.* 1996; 16:2397–410. [PubMed: 8601819]
51. Clapham DE. TRP channels as cellular sensors. *Nature.* 2003; 426:517–24. [PubMed: 14654832]
52. Fowler MA, Sidiropoulou K, Ozkan ED, Phillips CW, Cooper DC. Corticolimbic expression of TRPC4 and TRPC5 channels in the rodent brain. *PLoS One.* 2007; 2:e573. [PubMed: 17593972]

53. Pomrenze MB, Baratta MV, Rasmus KC, Cadle BA, Nakamura S, Birnbaumer L, Cooper DC. Cocaine self-administration in mice with forebrain knock-down of trpc5 ion channels. *F1000Res*. 2013; 2:53. [PubMed: 24358869]
54. Rasmus KC, O'Neill CE, Bachtell RK, Cooper DC. Cocaine self-administration in rats lacking a functional trpc4 gene. *F1000Res*. 2013; 2:110. [PubMed: 24555056]
55. Moreno E, Moreno-Delgado D, Navarro G, Hoffmann HM, Fuentes S, Rosell-Vilar S, Gasperini P, Rodriguez-Ruiz M, Medrano M, Mallol J, Cortes A, Casado V, Lluís C, Ferre S, Ortiz J, Canela E, McCormick PJ. Cocaine disrupts histamine H3 receptor modulation of dopamine D1 receptor signaling: sigma1-D1-H3 receptor complexes as key targets for reducing cocaine's effects. *J Neurosci*. 2014; 34:3545–58. [PubMed: 24599455]
56. Kourrich S, Hayashi T, Chuang JY, Tsai SY, Su TP, Bonci A. Dynamic interaction between sigma-1 receptor and Kv1.2 shapes neuronal and behavioral responses to cocaine. *Cell*. 2013; 152:236–47. [PubMed: 23332758]

Highlights

- Cocaine enhances IP₃-mediated Ca²⁺ signaling via sigma-1-receptor (σ_1 R)
- This induces TRP canonical (TRPC) activation and depolarization of accumbal neurons
- Intra-accumbal σ_1 R/TRPC blockade reduces cocaine-hyperlocomotion/sensitization.
- We show behavioral relevance of a novel cocaine-triggered pathway in the accumbens.

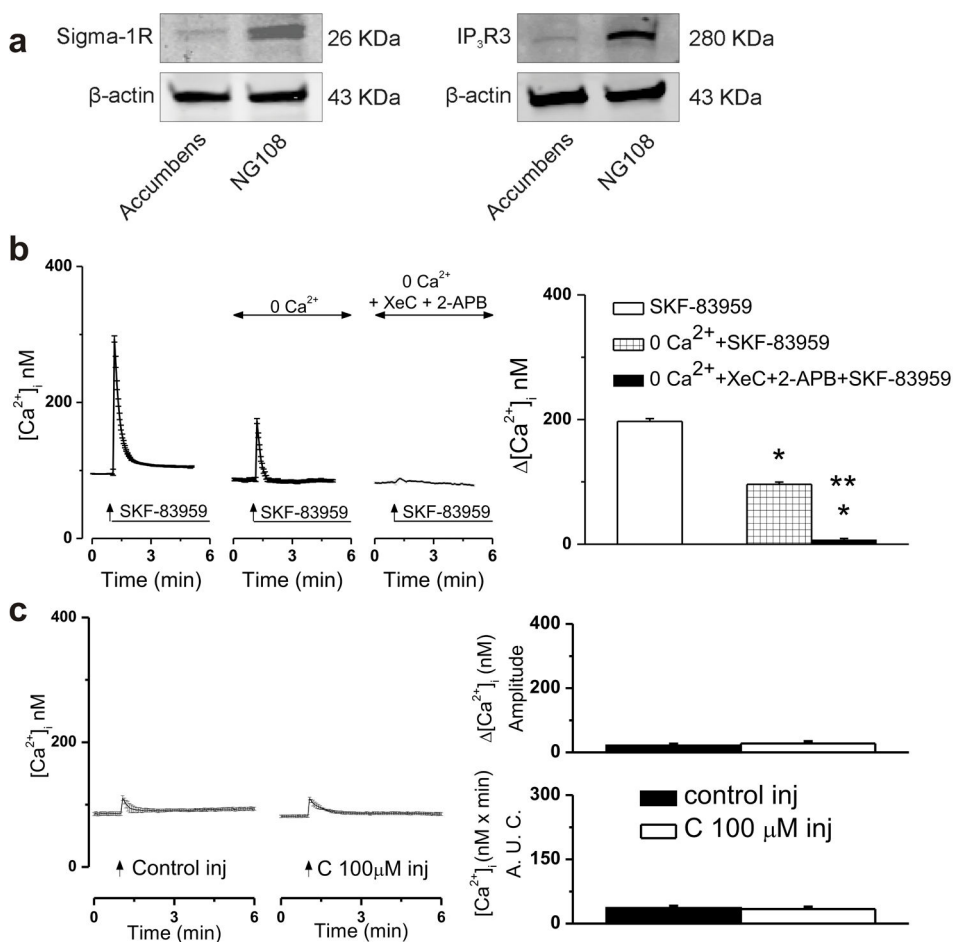


Figure 1. Neurons of interest

a, *Nucleus accumbens neurons express both σ_1 R and IP₃R3 proteins*; NG108 cells were used as positive control; β-actin was used as an internal control. Results are representative for three independent experiments. **b**, *Functional characterization of phosphatidylinositol-linked D₁ dopamine receptor expression in nAcc neurons*: left panel - averaged Ca^{2+} responses induced by D₁ agonist SKF83959 (10 μM) upon extracellular administration to D₁-expressing nAcc neurons incubated in Ca^{2+} -containing saline (left) or in Ca^{2+} -free saline, in absence (middle) and presence of IP₃R blockers xestospongine C (XeC) and 2-APB (right); right panel – comparison of the Ca^{2+} responses produced by SKF83959 in the mentioned conditions; $P < 0.00001$ compared to basal Ca^{2+} levels (*) or to SKF83959 in Ca^{2+} -free HBSS (**). Neurons not responding to SKF83959 with an increase in $[Ca^{2+}]_i$ were not used further for experiments. **c**, *Lack of effect of cocaine microinjection alone on $[Ca^{2+}]_i$ of D₁-positive nAcc neurons*: left - averaged tracings of the Ca^{2+} responses produced by intracellular microinjection of either control buffer or cocaine (C, 100 μM); right - Comparison of the amplitudes and areas under curve (A.U.C.) of the Ca^{2+} responses; lower concentrations of cocaine were similarly ineffective.

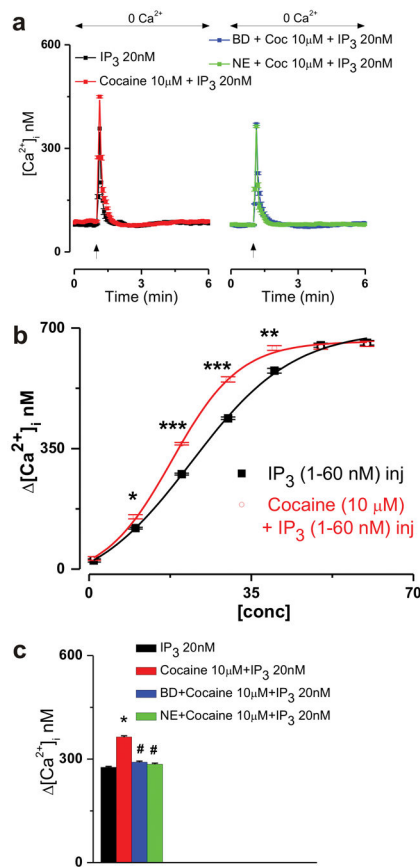


Figure 2. Cocaine shifts to the left the IP₃-induced concentration- Ca^{2+} response curve via $\sigma_1\text{R}$
a, Averaged Ca^{2+} responses elicited by IP₃ (20 nM) microinjection alone or in combination with cocaine (10 μM) or by IP₃ (20 nM) and cocaine (10 μM) coinjection upon $\sigma_1\text{R}$ blockade with either BD-1063 (BD, 10 μM) or NE-100 (NE, 3 μM). **b**, Concentration-response curves indicating the effect of IP₃ (1, 10, 20, 30, 40, 50 and 60 nM) microinjection into D₁-expressing nAcc neurons (incubated in Ca^{2+} -free saline) when injected alone (black) or in combination with 10 μM cocaine (red); $P < 0.005$ (*), $P < 0.001$ (**) and $P < 0.00001$ (***); comparison of the two data sets yielded statistical significance for the two fits: $F = 5.1715$; $P = 0.0378 < 0.05$. **c**, Comparison of the Ca^{2+} increases triggered by treatments mentioned in **a**; $P < 0.00001$ compared with IP₃ (*) or to combined cocaine and IP₃ microinjection (#).

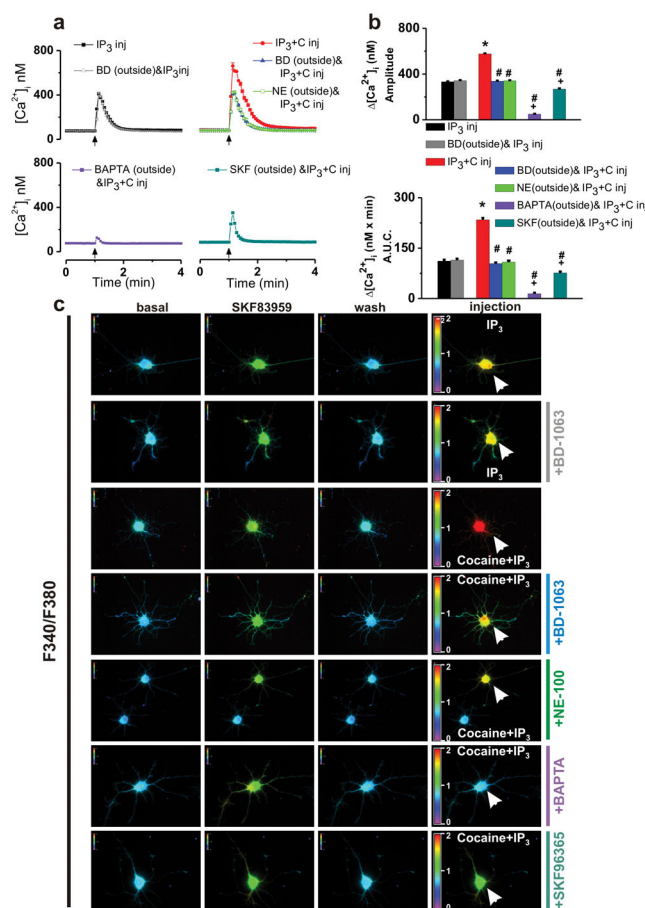


Figure 3. σ_1 R-mediated enhancement by microinjected cocaine of the IP₃-induced $[Ca^{2+}]_i$ increase

a, Averaged tracings of the Ca^{2+} responses elicited by intracellular microinjection of IP₃ (20 nM) alone or in presence of σ_1 R antagonist BD-1063 (BD, 10 μM); or cocaine (C, 10 μM) and IP₃ (20 nM) in absence, or presence of either BD (10 μM) or NE-100 (NE, 3 μM); the fast Ca^{2+} chelator BAPTA-AM (200 μM); or TRPC blocker SKF96365 (SKF, 2 μM). **b**, Comparison of the amplitudes and areas under curve (A.U.C.) of the Ca^{2+} responses; $P < 0.00001$ compared with IP₃ alone (*), with combined cocaine and IP₃ microinjection (#), or with all other treatment groups (+). **c**, Fura-2 AM fluorescence ratios (340 nm/380 nm) of cultured nAcc neurons before and after D₁ agonist SKF83959 (10 μM), after washing of SKF83959 and after microinjection of indicated compounds in absence and presence of the antagonist pretreatment indicated in the right side; cold colors indicate low levels of $[Ca^{2+}]_i$; hot colors indicate high levels of $[Ca^{2+}]_i$; fluorescence scale (0–2) is magnified in each panel showing the effect of injected compounds.

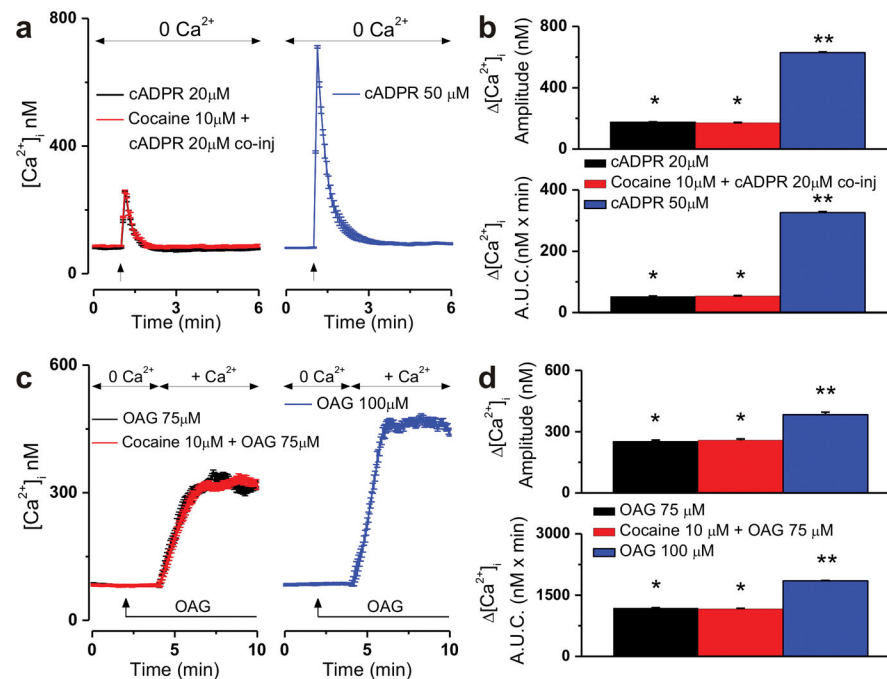


Figure 4. Cocaine does not enhance cADPR or OAG-mediated Ca^{2+} signaling in nAcc neurons
a, Averaged Ca^{2+} tracings indicating the response of Ca^{2+} -free saline-incubated neurons to microinjection of 20 μM cADPR alone or co-injected with 10 μM cocaine or to microinjection of 50 μM cADPR. **b**, Comparison of the amplitudes and areas under curve of the Ca^{2+} responses to treatments indicated in **a**; $P < 0.00001$ compared to basal Ca^{2+} levels (*) or to the effect of 20 μM cADPR (**). **c**, Averaged Ca^{2+} tracings corresponding to the effects produced by OAG (75 μM) in Ca^{2+} -free and Ca^{2+} -containing saline, in absence and presence of cocaine (10 μM), or to the effect of 100 μM OAG in Ca^{2+} -negative and Ca^{2+} -positive conditions. **d**, Comparison of the effects in Ca^{2+} -containing saline induced by treatments indicated in **d**; $P < 0.00001$ compared to basal Ca^{2+} levels (*) or to the effect of 75 μM OAG in presence of extracellular Ca^{2+} (**).

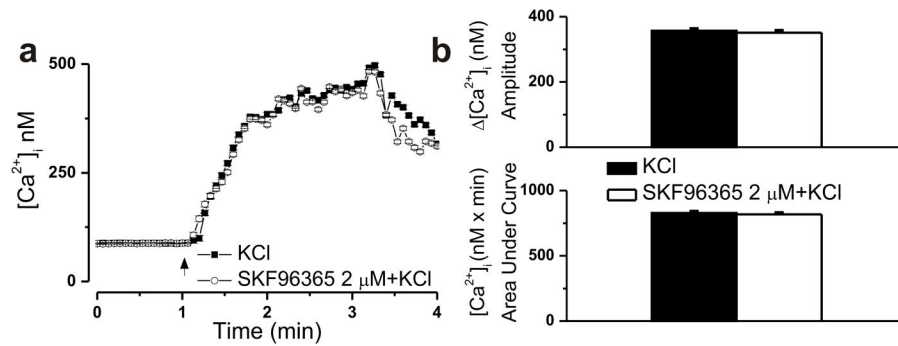


Figure 5. SKF96365 (2 μ M) is devoid of inhibitory activity at voltage-gated Ca^{2+} channels in nAcc neurons

a, Averaged Ca^{2+} responses induced by 30 mM KCl in absence and presence of SKF96365 (2 μ M). **b**, The amplitudes of the Ca^{2+} increases produced by KCl measured 357 ± 5.6 nM ($n = 31$ cells) in absence and 351 ± 5.9 nM ($n = 37$) in presence of SKF96365; the areas under curve were 828 ± 11.3 nM x min and 817 ± 10.9 nM x min, respectively.

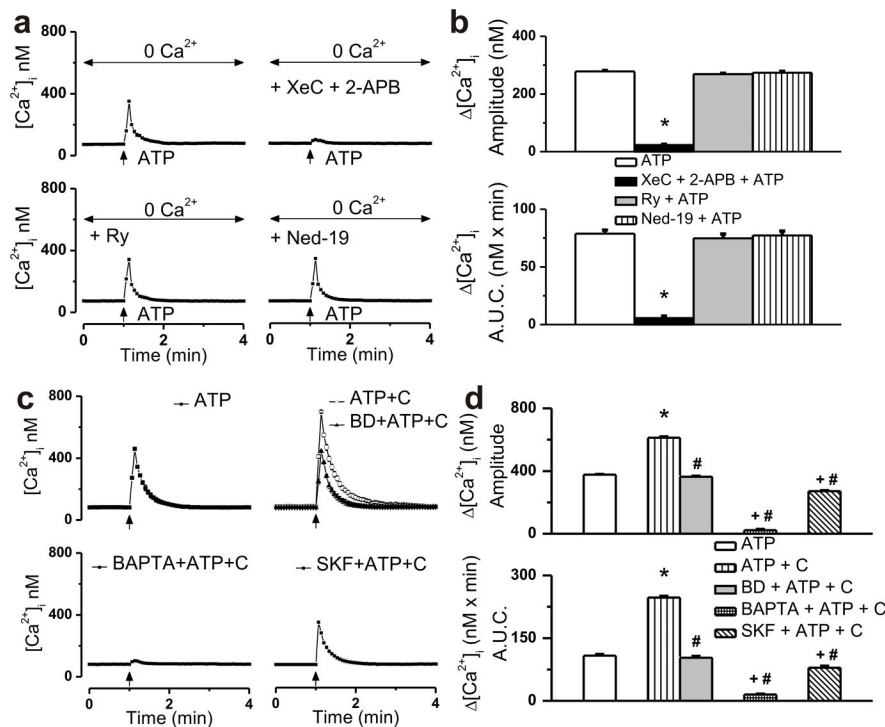


Figure 6. Cocaine potentiates the Ca^{2+} response of nAcc neurons to IP_3 -generating molecules
a–b, ATP promotes Ca^{2+} mobilization from IP_3 -sensitive pools in accumbens neurons: **a**, Averaged tracings of the Ca^{2+} responses elicited by ATP (20 μ M) in Ca^{2+} -free saline, in absence or presence of IP_3 R blockers xestospongine C (XeC) and 2-aminoethoxydiphenyl borate (2-APB); ryanodine receptor blocker ryanodine (Ry); or lysosomal two-pore channel inhibitor Ned-19. **b**, Comparison of the amplitudes and the areas under curve (A.U.C.) of the Ca^{2+} increases produced by the indicated treatments; * $P < 0.00001$ compared with all other treatment groups. **c–d, Cocaine produces σ_1 R-dependent potentiation of the ATP-induced Ca^{2+} elevation in D_1 -expressing accumbens neurons:** **c**, Averaged tracings of the Ca^{2+} responses induced by bath application of ATP (20 μ M) or cocaine (C, 10 μ M) and ATP (20 μ M), in naïve neurons or neurons incubated with either σ_1 R antagonist BD-1063 (BD, 10 μ M); or Ca^{2+} chelator BAPTA-AM (200 μ M); or TRPC blocker SKF96365 (SKF, 2 μ M). **d**, Comparison of the amplitudes and the areas under curve (A.U.C.) of the Ca^{2+} responses produced by the indicated treatments; $P < 0.00001$ compared with ATP alone (*), with combined cocaine and ATP administration (#), or with all other treatment groups (+).

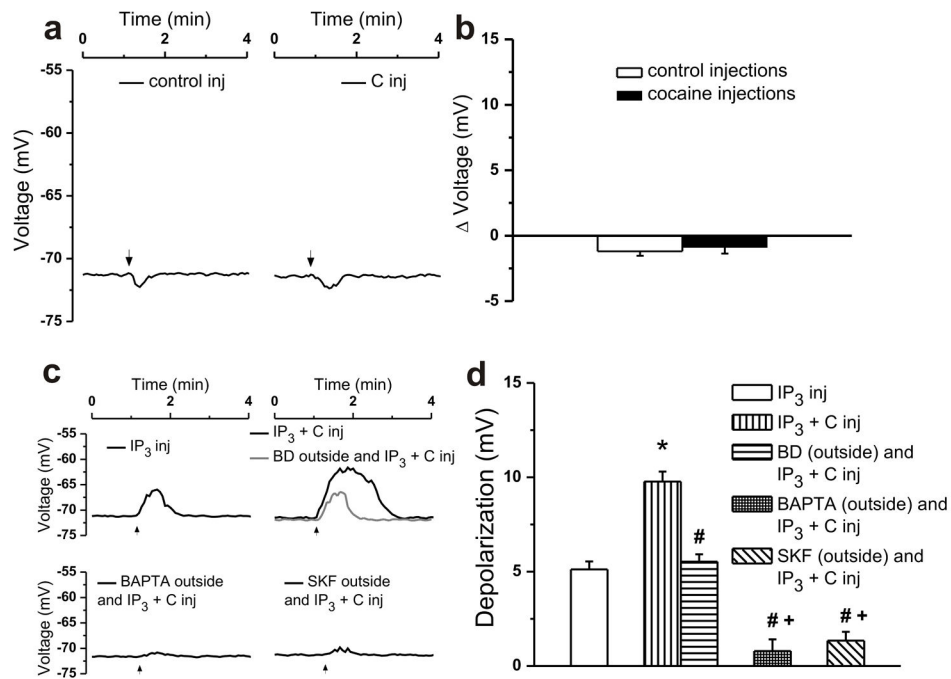


Figure 7. Intracellular microinjection of cocaine enhances IP₃-induced depolarization in the nAcc

a–b, Cocaine microinjection alone does not modify neuronal membrane potential: **a**, Representative recordings of the resting membrane potential of D₁-expressing nAcc neurons treated intracellularly with either control vehicle or cocaine (C, 10 μ M, final intracellular concentration). **b**, Neither control vehicle microinjection or cocaine microinjection did produce a significant change in neuronal membrane potential. **c–d, Cocaine potentiates the IP₃-dependent depolarization of D1-expressing accumbens neurons:** **c**, Characteristic changes in resting membrane potential of neurons microinjected with either IP₃ (20 nM) or IP₃ (20 nM) and cocaine (C, 10 μ M) in absence and presence of bath-applied σ_1 R inhibitor BD-1063 (BD, 10 μ M), of the Ca²⁺ chelator BAPTA-AM (200 μ M) or of TRPC blocker SKF96365 (SKF, 2 μ M). **d**, Comparison of the amplitudes of the depolarizations induced by treatment conditions described in **c**; $P < 0.00001$ compared with IP₃ injection alone (*), with combined cocaine and IP₃ microinjection (#), or with all other treatment groups (+).

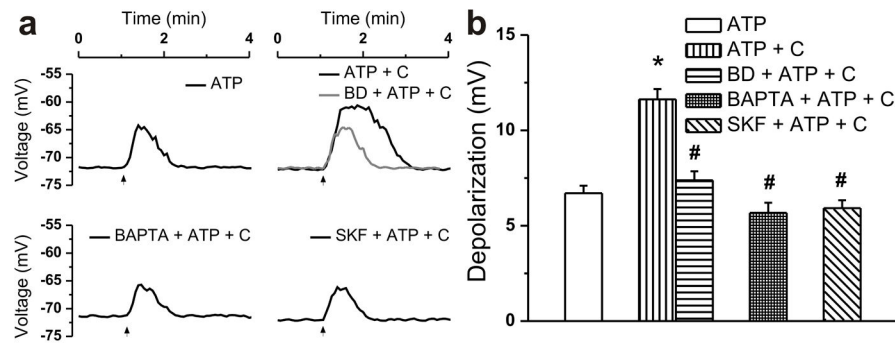


Figure 8. Cocaine enhances the ATP-induced depolarization

a, Typical recordings of membrane potential modifications of D1-expressing accumbens neurons treated extracellularly with ATP (20 μ M) or ATP (20 μ M) and cocaine (C, 10 μ M) in absence and presence of the σ_1 R inhibitor BD-1063 (BD, 10 μ M), of the Ca^{2+} chelator BAPTA-AM (200 μ M) or of the TRPC blocker SKF96365 (SKF, 2 μ M). **b**, Comparison of the amplitudes of the depolarizations induced by treatments described in **a**; $P < 0.00001$ compared with ATP alone (*) or with combined cocaine and ATP administration (#).

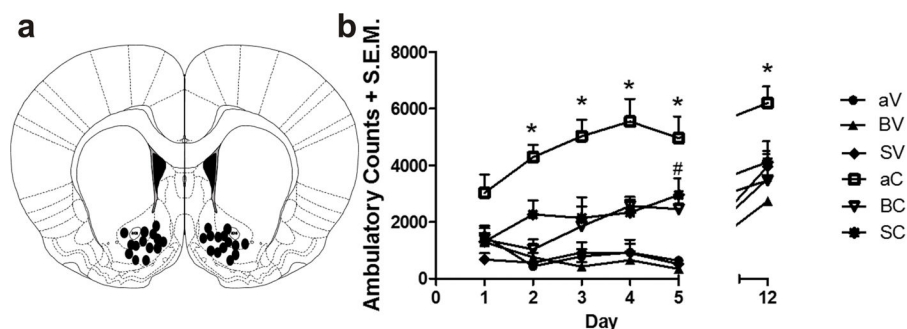


Figure 9. Cocaine-induced behavioral hyperactivity and sensitization involves σ_1 R and TRPC
a, Coronal section indicating the distribution of infusion sites in the nAcc for the 35 experimental animals (aCSF-saline, n = 5; aCSF-cocaine, n = 7; BD-1063-saline, n = 5; BD-1063-cocaine, n = 6; SKF96365-saline, n = 6; SKF96365-cocaine, n = 6). Injection sites may appear fewer than the reported number of rats because of overlap of placements. **b**, Pretreatment with BD-1063 (80 μ g/side) or SKF96365 (20 μ g/side) administered 20 min prior to each cocaine injection for 5 days significantly inhibited cocaine-induced ambulatory activity on Days 2–5, and the development of repeated cocaine-induced locomotor sensitization on Day 12. Repeated administration of either antagonist alone did not alter levels of locomotion; * $P < 0.05$, aCSF-cocaine rats were significantly different from all other experimental groups; # SKF96365-cocaine rats were significantly different from aCSF-saline, BD-1063-saline and SKF96365-saline on day 5. Data are presented as mean \pm sem ambulatory counts/60 minutes; abbreviations: aV, intra-nAcc aCSF + ip saline vehicle; BV, intra-nAcc BD-1063 + ip saline vehicle; SVm intra-nAcc SFK96365 + ip saline vehicle; aC, intra-nAcc aCSF + ip cocaine; BC, intra-nAcc BD1063 + ip cocaine; SC, intra-nAcc SKF-96365 + ip cocaine.

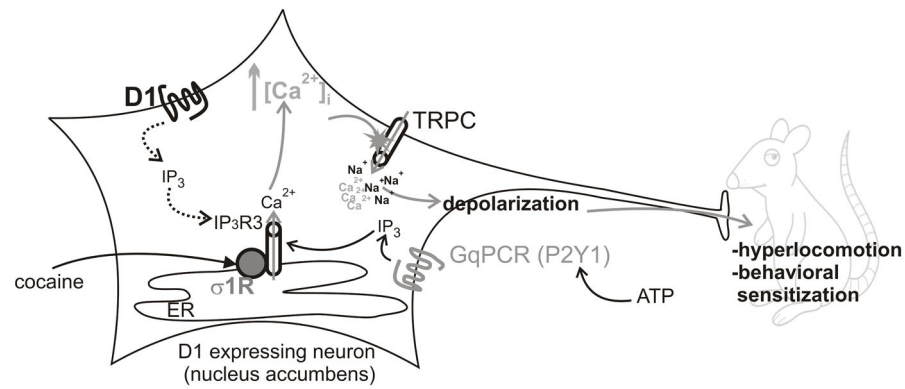


Figure 10. Mechanism of cocaine-induced activation of D₁-expressing nAcc neurons

Cocaine activates endoplasmic reticulum (ER)-located σ_1R s and potentiates Ca^{2+} release from the ER via IP_3 receptors type 3 (IP_3R3) promoted by GPCR agonists (Gq-coupled, such as ATP). The increase in cytosolic Ca^{2+} triggers activation of TRPC and additional Ca^{2+} entry, as well as Na^+ entry, followed by depolarization and activation of these neurons, triggering hyperlocomotion and behavioral sensitization *in vivo*.