Supplementary Information

Role of RGS12 in the differential regulation of kappa opioid receptor-dependent signaling and behavior

Joshua D. Gross [1,2,3], Shane W. Kaski [1,2,3], Karl T. Schmidt [4], Elizabeth S. Cogan [4], Kristen M. Boyt [4], Kim Wix [1], Adam B. Schroer [1], Zoe A. McElligott [4], David P. Siderovski [1,2*], and Vincent Setola [1,2,3]

[1] Department of Physiology & Pharmacology,
[2] Department of Neuroscience, and
[3] Department of Behavioral Medicine & Psychiatry,
West Virginia University, Morgantown WV 26506-9229 USA;
[4] Bowles Center for Alcohol Studies and Department of Psychiatry,
University of North Carolina at Chapel Hill, Chapel Hill NC 27599 USA

Supplementary Methods

β-Arrestin-2 deficient animals

Male and female β-arrestin-2 knockout ($\beta arr2^{KO}$) (JAX Stock 023852) and wildtype C57BL/6 control mice (JAX Stock 000664) were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under identical conditions to RGS12-null and wildtype littermates.

Synaptosomal [^{3}H]DA uptake

Synaptosomal [^{3}H]DA uptake was performed as described [1] with minor modifications. Briefly, RGS12-null mice and wildtype littermates were administered vehicle (0.9% NaCl) or *nor*-BNI (10 mg/kg, ip) 24 hours prior to dissection. Ventral striata were then extracted and synaptosomes prepared as described [1]. Synaptosomal fractions were incubated with 0.8 μM DA (with [^{3}H]DA content diluted to 8 nM final concentration) in Krebs-Ringer buffer [1] for 10 minutes. Non-specific uptake was determined in the presence of DAT-selective reuptake inhibitor GBR12935. Uptake reactions were terminated by filtration

over Whatman GF/B filters with ice-cold Krebs-Ringer buffer, washed three times, and measured via liquid scintillation counting. [^{3}H]DA uptake analyses in tissue samples from βarr2^{KO} and wildtype control mice were performed identically to RGS12-null and wildtype littermates, except that these mice did not receive vehicle or *nor*-BNI pretreatment before testing.

Locomotor activity

AMPH-induced locomotor activity was measured as described [35] with the following modifications: RGS12-null mice and wildtype littermates received vehicle (0.9% NaCl) or *nor*-BNI (10 mg/kg, ip) 24 hours prior to quantification of AMPH-induced hyperlocomotion. For U50,488-induced locomotion, RGS12-null mice and wildtype littermates were administered vehicle (0.9% saline, ip) or U50,488 (2.5 or 5 mg/kg, ip), immediately placed into open-field chambers (San Diego Instruments), and X,Y coordinate beam breaks measured in five-min bins for 30 minutes.

Fast-scan cyclic voltammetry (FSCV)

RGS12-null and wildtype littermates were injected with 10 mg/kg *nor*-BNI or saline (i.p.). 24 hours later, brain slices were prepared and FSCV performed as described [2]. Briefly, mice under deep anesthesia were decapitated, and brain tissue was submerged in oxygenated ice-cold sucrose. 300 μm slices were cut with a Leica VT1000 vibratome and placed in oxygenated artificial cerebral spinal fluid (aCSF) [2] for at least 30 min prior to electrochemical testing. Striatal slices were transferred to an electrochemistry rig and perfused at 2 mL/min with oxygenated aCSF. Slices were stimulated at 100, 200, and 300 μA with a bipolar nichrome electrode (single pulse, 4 ms duration). DA signals were recorded with an in house fabricated 100 µm x 7 µm carbon fiber microelectrode placed ~ 100 um from the stimulating electrode [3]; a custom built potentiostat (University of

Washington, Seattle) and TarHeel CV written in LabVIEW (National Instruments) were used to apply a triangle waveform (-0.4 to 1.3 V) cycling at 10 Hz. Cyclic voltammograms (CVs) were analyzed with HDCV (UNC Chapel Hill) to create IvT (current *vs* time) plots that were analyzed with Clampfit 10.6 software (Molecular Devices, Sunnyvale, CA).

Radioligand binding assays

[^{3}H]WIN35428 binding to DAT

Evaluation of DAT binding sites was performed as described [35], with minor modifications. As KOR-mediated changes to DAT levels requires prolonged drug treatment [43], mice were treated with vehicle or the long-lasting, irreversible KOR antagonist *nor*-BNI (10 mg/kg, saline, ip) for 14 days [47] before DAT binding was assessed. Mice received two injections of *nor*-BNI, first on day 0 and then on day 7. On day 14, mice were sacrificed, vSTR microdissected, and extracts frozen on dry ice. Crude membrane fractions were prepared and incubated with [^{3}H]WIN35428 (52.5 nM) in the presence or absence of 10 μM GBR12935 to determine DAT-specific binding; as we previously found that RGS12-null mice do not exhibit complete DAT saturation in assays of vSTR DAT binding [35], therefore, the relative number of DAT binding sites was determined using a single, high concentration of [^{3}H]WIN35428 (52.5 nM), as previously described [35]. Binding reactions proceeded for 1 hour at RT and then terminated by rapid filtration over PEI (0.5%)-soaked Whatman GF/B filters with ice-cold Tris buffer. Protein content-adjusted data (in fmol/mg protein) were normalized to the wildtype vehicle condition and expressed as fold change to control for variability in the efficacy of [^{3}H]WIN35428 binding to DAT across experiments. Data are displayed as mean ± SEM across multiple experiments and statistical analyses performed with two-way ANOVA followed by Sidak's post-hoc test (n = 6 mice per group).

[^{3}H]U69,593 binding to KOR and [^{35}S]GTPγS incorporation by KOR activation

KOR saturation binding and GTPγS incorporation assays were performed as described [48] with modifications. Ventral and dorsal striatal brain regions were dissected, immediately frozen on dry ice, homogenized with a polytron homogenizer in 10 volumes of buffer (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT), and passed through a 27-gauge needle eight times. Homogenates were centrifuged twice at 20,000 x *g* for 30 min; pellets were resuspended in KOR assay buffer: either 50 mM Tris-HCl pH 7.4 for [^{3}H]U69,593 binding, or 50 mM Tris-HCl pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 20 μM GDP, 1 mM DTT for GTPγS incorporation. For saturation binding, resuspensions were added to [^{3}H]U69,593 (from 0.63 - 20 nM) and incubated for 2 hr at room temperature. Nonspecific binding was determined in the presence of 10 μM *nor*-BNI. For GTPγS incorporation, resuspensions were added to [^{35}S]GTPγS (0.1 nM) and several independent concentrations of the KOR agonist U50,488 (0.0001, 0.01, 1, and 100 μM; dissolved in saline). Binding was terminated by filtration over Whatman GF/B filters with ice-cold deionized water (washed three times) and measured via liquid scintillation counting after overnight drying.

Co-immunoprecipitations

Co-immunoprecipitation (co-IP) analyses in brain tissue were performed with the Pierce co-IP Kit (Waltham, MA) according to manufacturer's instructions with minor modifications. Briefly, anti-RGS12 (UNC60-26.2.1; DSHB, Iowa City, IA) was immobilized on the kit's AminoLink Plus-coupled resin. Ventral striata were dissected, immediately frozen on dry ice, and then homogenized with a polytron homogenizer in co-IP lysis buffer (Pierce), followed by addition of CHAPS detergent (1% final concentration). Homogenates were incubated for 1 hr at 4°C (with rotation) to facilitate KOR solubilization [49]. Solubilized lysates were precleared with quenched resin and then added to the anti-

KOR-immobilized resin for overnight incubation (4°C with rotation). Immunoprecipitates were washed, eluted, and denatured with Laemmli buffer containing 100 mM DTT (Pierce) for 30 minutes at 37°C. IP and whole lysate (input) samples were then subjected to SDS-PAGE, transferred to nitrocellulose, and incubated overnight at 4°C with anti-KOR (#AOR-012; Alomone Labs, Jerusalem, Israel), a control mixture of anti-KOR plus blocking peptide (AOR-012 immunizing peptide/control antigen; Alomone), or anti-RGS12. Immunoblots were then washed, incubated with the appropriate HRP-conjugated secondary antibodies for 1 hr at RT, and visualized with enhanced chemiluminescence.

Co-IP analyses in HEK293T cells were performed with the Pierce HA-Tag IP/Co-IP Kit according to manufacturer's instructions with minor modifications. Briefly, HEK293T cells were transiently transfected (via $CaPO_4$ [50]) with wildtype human RGS12 (hRGS12-WT) alone or co-transfected with hRGS12-WT and 3XHA-hKOR or 3XHA-hMOR HA-tagged human opioid receptors (www.cdna.org). Fourty-eight hours later, transfected cells were lysed with co-IP lysis buffer (Pierce), CHAPS added (1% final concentration), and lysates solubilized for 1 hr (4°C with rotation). Solubilized lysates were then added to pre-coupled anti-HA agarose spin columns and incubated overnight (4°C with rotation) to immunoprecipitate 3XHA-KOR or 3XHA-MOR proteins. Immunoprecipitates were washed, eluted, and denatured in 2X Sample Buffer with 100 mM DTT (Pierce). IP and whole lysate (input) samples were then subjected to SDS-PAGE, transferred to nitrocellulose, and incubated overnight at 4°C with anti-RGS12 (sc-398545, Santa Cruz, Dallas, TX) or anti-HA (Cat. 3725, Cell Signaling Technology, Danvers, MA). Immunoblots were then washed, incubated with the appropriate HRP-conjugated secondary antibodies for 1 hr at RT, and visualized with enhanced chemiluminescence.

cAMP inhibition

HEK293T cells were transfected *via* $CaPO_4$ [50] with vectors expressing GloSensor-22F cAMP biosensor (Promega) and HA-tagged human opioid receptor (3XHA-hKOR, 3XHA-hMOR, or 3XHA-hDOR; www.cdna.org), with or without wildtype human RGS12, RGS domain loss-of-function RGS12 (*Rgs12*^{E740K}), or GoLoco motif loss-of-function RGS12 (*Rgs12*^{R1206F}). Twenty-four hours later, transfected cells were seeded onto white, clear bottom, 96-well plates (8 x 10^6 cells/plate). Next morning, cells were incubated with D-luciferin in buffer (20 mM HEPES, 140 mM NaCl, 5 mM KCl 250 μM $NaPO_4$, 0.1 mM glucose, 0.5 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM $NaHCO_3$, pH 7.4) for 2 hours at room temperature. Cells were then treated with various agonist concentrations (U50,488 for KOR, DAMGO for MOR, and DADLE for DOR) for 15 minutes at RT. Then, cells were treated with isoproterenol for 15 minutes to stimulate G_s signaling and adenylyl cyclase activation. Luminescence was measured with a FlexStation3 plate-reader (Molecular Devices). In pertussis toxin (PTX) pre-treatment assays, to uncouple $G_{i/o}$-coupled GPCRs via Gα ADP-ribosylation [51,52], cells were pretreated with 200 ng/mL PTX (or vehicle) for 24 hours [53] prior to luminescence measurement.

β-arrestin recruitment

Agonist-induced β-arrestin recruitment was determined using 'Tango' assay [54,55]. HTLA cells, stably expressing β-arrestin-TEV protease fusion and tetracycline transactivator-driven luciferase reporter [54], were transiently transfected *via* $CaPO_4$ [50] with opioid receptor-Tango vectors (FLAG-KOR-Tango, FLAG-MOR-Tango, or FLAG-DOR-Tango; contributed to Addgene by Dr. Bryan Roth) with or without WT hRGS12, *Rgs12*^{E740K}, or *Rgs12*^{R1206F} vectors. Twenty-four hours later, transfected cells were seeded onto white, clear bottom, 384 well plates (8 x 10^6 cells/plate). Next morning, cells

were serum starved for 6 hours, then stimulated with various agonist concentrations (U50,488 for KOR, DAMGO for MOR, or DADLE for DOR). The following morning, cells were lysed with BrightGlo (Promega) and luminescence measured with a FlexStation3. In Tango assays performed with PTX, cells were pretreated with 200 ng/mL PTX or vehicle 24 hours prior to lysis and luminescence measurement.

In silico single cell RNAseq analysis

The DropViz single cell RNAseq database [4] was analyzed to determine the mouse brain neuronal cell populations that co-express *Rgs12* and *Oprk1* mRNA. Data displayed are the output (*Table*) derived from a query with *Rgs12* and *Oprk1* (*Gene*) and *Neuron* (*Limit by Class*) set as query parameters. Results were sorted by the neuronal single cell populations expressing the highest levels of *Oprk1* (*Oprk1 Amount*) and constrained to corticostriatal circuitry (frontal cortex, striatum, substantia nigra, globus pallidus, and thalamus) given the relevance of these regions to the behavioral effects of KOR agonism [5-7].

In situ hybridization

In situ hybridization on mouse brain slices was performed as previously described [1]; dual-label detection of *Rgs12* and *Oprk1* expression in the claustrum was performed by the RNA In Situ Hybridization Core at Baylor College of Medicine, which is, in part, supported by a Shared Instrumentation grant from the NIH (1S10OD016167).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Ventral striatum (vSTR) and midbrain were rapidly dissected, immediately frozen on dry ice, and then homogenized on ice in TRIzole reagent (Invitrogen™). RNA was extracted according to manufacturer's instructions (Direct-zol RNA MiniPrep, Zymo); genomic DNA was eliminated with the QuantiTech Reverse Transcription Kit (Qiagen). RNA quantity was assessed by QIAexpert bioanalyzer (Qiagen) and cDNA synthesized with

QuantiTech Reverse Transcription (Qiagen). Published primers [8] for *Oprk1*/*KOR* (F: 5'-TCCCCAACTGGGCAGAATC-3', R: 5'-GACAGCGGTGATGATAACAGG-3') and *Pdyn* (F: 5'- CAGTGAGGATTCAGGATGGG-3', R: 5'-CGTCAGGGTGAGAAAAGATGA-3') were obtained from Invitrogen. Data were normalized to averaged Ct values from three housekeeping genes: β-Actin (F: 5'-GGCTGTATTCCCCTCCATCG-3', R: 5'-CCAGTTGGTAACAATGCCATGTT-3'), 18S rRNA (Qiagen), and β-Tubulin (F: 5'-GGGAGGTGATAAGCGATGA-3', R: 5'-CCCAGGTTCTAGATCCACCA). Two μL of cDNA template was used in each qRT-PCR reaction with SYBR Green PCR Master Mix (Qiagen). qRT-PCR was performed with a Qiagen Rotor Gene-Q; melt curves were analyzed after each run to ensure primer specificity.

Hot plate analgesia test

To detect sensitivity to painful stimuli, the hot plate test of nociception was performed as previously described [56] with slight modifications. Mice were placed on a hot plate (53 oC) and prevented from escape by a tall plastic cylinder. Latency to lick a hindlimb, flinch/flick, jump, or other first sign of nociception was recorded. On day 1, RGS12-null mice and wildtype littermates were administered saline (0.9% NaCl) 30 minutes prior to the test and nociceptive latency recorded. On day 2, all mice were administered U50,488 (5, 22.5, or 30 mg/kg, ip) 30 minutes prior to the test and nociceptive latency was recorded.

Hot plate analgesia with KOR antagonist treatment

Hot plate analgesia assays with *nor*-BNI-treated mice were performed identically to all other hot plate analgesia assays (Fig. 4A-C) except that mice were administered *nor*-BNI (10 mg/kg, ip, dissolved in 0.9% NaCl) following their baseline measurement; thus, mice underwent a 24 hour pretreatment of *nor*-BNI prior to the administration of 30 mg/kg U50,488 (ip) and subsequent hot plate latency testing.

Conditioned place aversion (CPA)

CPA was performed as described [3] with modifications denoted below. Mice were free to acclimate to a two-chambered place preference apparatus (Med Associates) on day 1 for 30 min; time spent in each compartment was recorded to determine the most-preferred chamber (preconditioning). In the morning of days 2 and 3, all RGS12-null mice and wildtype littermates were administered saline (0.9% NaCl) and confined to their least-preferred chamber. In the afternoon of days 2 and 3, all mice received U50,488 (2.5 or 5.0 mg/kg, ip) and were confined to their most-preferred chamber for 30 min. On day 4 (postconditioning), CPA was determined by allowing mice to freely explore. Pre- and post (U50,488)-conditioning were computed by subtracting the time spent (seconds) in the most-preferred chamber (drug-paired during conditioning) minus the least-preferred chamber (saline-paired during conditioning).

Supplementary Figure Legends

Figure S1. *Rgs12* and *Oprk1 (KOR)* mRNAs show similar expression patterns in mouse brain. Coronal *in situ* hybridization (ISH) from the Allen Brain Atlas highlighting coincident *Oprk1* (**A**) and *Rgs12* (**B**) mRNA expression in hippocampus (HPC), and the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), with the latter two regions being beds of mesolimbic and nigrostriatal dopaminergic soma, respectively. (**C**) *In silico* analysis of a single cell RNAseq dataset (DropViz) shows co-expression of *Rgs12* with *Oprk1* (*KOR*) in neuronal cell populations across several brain regions. Data are sorted and displayed by the neuronal cell populations expressing the highest levels of *Oprk1* (***Oprk1 amount***). *Rgs12* exhibits co-expression with *Oprk1* in many of the top *Oprk1*-expressing neurons, particularly within brain regions composing the corticostriatal circuit (*e.g.*, frontal cortex, striatum, substantia nigra, globus pallidus, thalamus). The highest expressing neuronal cell type for both *Rgs12* and *Oprk1* are *Nr4a2+* cells within the claustrum (a subregion of the frontal cortex), as observed in dual-label ISH below. (**D-F**) Epifluorescence images of dual-label ISH of a coronal brain slice of a wildtype mouse with antisense RNA probes directed to *Oprk1* mRNA (panel **D**, *green*) and *Rgs12* mRNA (panel **E***, red*); overlay of both signals is presented in *yellow* in panel **F** (CLA, claustrum; CTX, cortex; ec, external capsule; STR, striatum). Parallel dual-label ISH of a brain slice from an RGS12-null (*Rgs12*$^{-/-}$) mouse resulted in no signal in the red channel (*data not shown*).

Figure S2. Baseline and KOR-dependent DA release and DA reuptake are normal in the dSTR of RGS12-null mice. (**A**) Electrically-stimulated DA release (peak DA current, nA) and (**B**) DA reuptake ($t_{1/2}$) in the dSTR of brain slices from RGS12-null and wildtype mice pretreated with vehicle (saline) or *nor*-BNI (10 mg/kg, ip) 24 hours prior to FSCV measurements. Analyses reveal an effect of stimulation amplitude on DA release ($p < 0.0001$); however, no genotype, treatment, or interaction effects were observed in measures of DA release or DA reuptake ($p > 0.05$). All FSCV data are mean ± SEM (n = 6 - 11 mice per group) and were analyzed by general linear mixed model ANOVA (ns, not significantly different).

Figure S3. *Oprk1* and *prodynorphin* mRNA levels are unchanged in the vSTR and midbrain of RGS12-null mice. (**A**) Measurement of *Oprk1* (*KOR*) mRNA levels in ventral striatum (vSTR) and midbrain by qRT-PCR. RGS12-null and wildtype mice exhibited similar *Oprk1* mRNA levels in both regions (*omnibus*: genotype, $F(1,20) = 0.2$, $p = 0.624$; region, $F(1,20) = 0.4$, $p = 0.567$; interaction, $F(1,20) = 0.4$, $p = 0.536$) (*multiple comparisons*: *Oprk1*-vSTR: RGS12-null vs wildtype: $p = 0.994$, *Oprk1*-midbrain: RGS12-null vs wildtype: $p = 0.680$). (**B**) Measurement of dynorphin precursor prodynorphin (*Pdyn*) mRNA levels in vSTR and midbrain by qRT-PCR. Analysis revealed that prodynorphin mRNA levels in vSTR and midbrain were comparable between RGS12-null mice and wildtype littermates (*omnibus*: genotype, $F(1,19) = 0.02$, $p = 0.885$; region, $F(1,19) = 1.4$, $p = 0.255$; interaction, $F(1,19) = 0.9$, $p = 0.351$) (*multiple comparisons*: *Pdyn*-vSTR: RGS12-null *vs* wildtype: $p = 0.809$, *Pdyn*-midbrain: RGS12-null *vs* wildtype: $p = 0.703$). All data are the mean ± SEM and tested by two-way ANOVA with Sidak's post hoc test (n = 6 mice per group).

Figure S4. The selective effect of RGS12 on KOR-mediated G protein signaling is preserved in rodent RGS12 variants. Glo-Sensor luciferase-based measurements of cAMP levels in HEK293T cells stimulated with 100 nM isoproterenol and simultaneously inhibited with indicated receptor-selective agonists following transient co-expression (**A**) 3XHA-hKOR or (**B**) 3XHA-hMOR cDNA plus empty vector, untagged wildtype mouse RGS12 (mRGS12), or myc-tagged wildtype rat RGS12 (rRGS12) expression plasmids. Co-expression of mRGS12 or rRGS12 each reduced KOR agonist potency by ~30-fold (Fig. S4A) (*mRGS12*: $F(1, 56) = 14.1$, $p = 0.0004$; KOR + mRGS12 $pIC_{50} = 8.1 \pm 0.2$ *vs* KOR only $pIC_{50} = 9.6 \pm 0.1$) (*rRGS12*: $F(1, 56) = 12.0$, $p = 0.001$; KOR + rRGS12 $pIC_{50} = 8.1 \pm 0.2$ *vs* KOR only $pIC_{50} = 9.6 \pm 0.1$). In contrast, mRGS12 and rRGS12 each attenuated MOR agonist potency by only ~6-fold (Fig. S4B) (*mRGS12*: $F(1, 56) = 10.7$, $p = 0.001$; MOR + mRGS12 $pIC_{50} = 7.1 \pm 0.1$ *vs* MOR only $pIC_{50} = 7.9 \pm 0.1$) (*rRGS12*: $F(1, 56) = 7.5$, $p = 0.008$; MOR + $rRGS12^{WT}$ $pIC_{50} = 7.1 \pm 0.2$ *vs* MOR only $pIC_{50} = 7.9 \pm 0.1$). Data were normalized to vehicle control conditions and are expressed as the mean ± SEM from multiple experiments. Concentration-response curves were fit by four-parameter non-linear regression (Prism 7).

Figure S5. The analgesic effect of 30 mg/kg U50,488 is lost, in both RGS12-null mice and wildtype littermate controls, following pretreatment with *nor*-BNI. U50,488 (30 mg/kg)-induced supraspinal analgesia is KOR-dependent. Omnibus ANOVA analysis revealed no effects of genotype, drug treatment (U50,488 + *nor*-BNI), or genotype x drug treatment ($p > 0.05$). Data are the mean ± SEM (n = 8-10 mice per group) and tested by two-way ANOVA.

Figure S6. Responses to morphine at doses of either 5 mg/kg (A) or 10 mg/kg (B) are similar in RGS12-null mice and wildtype littermate controls. (**A**) Omnibus ANOVA analysis revealed no effects of genotype, morphine treatment, or genotype x drug treatment ($p > 0.05$). (**B**) Omnibus ANOVA analysis revealed only an effect of morphine treatment ($F(1,27) = 29.1$, $p < 0.001$); *multiple comparisons*: RGS12-null saline *vs* 10 mg/kg morphine: $p = 0.0005$; wildtype saline *vs* 10 mg/kg morphine: $p = 0.004$. Data are the mean ± SEM (n = 7-14 mice per group) and tested by two-way ANOVA followed by Sidak's *post hoc* test (**, $p < 0.01$; ***, $p < 0.001$).

Figure S7. β-arrestin-2 is not required for [^{3}H]DA uptake in the vSTR and dSTR. Uptake of [^{3}H]DA (8 nM) in synaptosomes prepared from vSTR and dSTR brain tissue from βarr2^{KO} and wildtype mice. Non-specific binding was determined in the presence of 10 μM GBR12935. Data shown are the mean ± SEM (n = 4 mice per group) from a representative experiment and tested by two-way ANOVA ($p > 0.05$). [^{3}H]DA uptake in the vSTR and dSTR of βarr2^{KO} and wildtype mice was performed twice (n = 8 mice per group over 2 independent experiments).

Literature Cited in Supplementary Information

1. Gross JD, Kaski SW, Schroer AB, Wix KA, Siderovski DP, Setola V. Regulator of G protein signaling-12 modulates the dopamine transporter in ventral striatum and locomotor responses to psychostimulants. J Psychopharmacol 2018;32:191-203.

2. Schmidt KT, Makhijani VH, Boyt KM, Cogan ES, Pati D, Pina MM*, et al*. Stress-Induced Alterations of Norepinephrine Release in the Bed Nucleus of the Stria Terminalis of Mice. ACS Chem Neurosci 2018.

3. Kile BM, Walsh PL, McElligott ZA, Bucher ES, Guillot TS, Salahpour A*, et al*. Optimizing the Temporal Resolution of Fast-Scan Cyclic Voltammetry. ACS Chem Neurosci 2012;3:285-292.

4. Saunders A, Macosko EZ, Wysoker A, Goldman M, Krienen FM, de Rivera H*, et al*. Molecular Diversity and Specializations among the Cells of the Adult Mouse Brain. Cell 2018;174:1015-1030 e1016.

5. Tejeda HA, Wu J, Kornspun AR, Pignatelli M, Kashtelyan V, Krashes MJ*, et al*. Pathway- and Cell-Specific Kappa-Opioid Receptor Modulation of Excitation-Inhibition Balance Differentially Gates D1 and D2 Accumbens Neuron Activity. Neuron 2017;93:147-163.

6. Trifilieff P, Martinez D. Kappa-opioid receptor signaling in the striatum as a potential modulator of dopamine transmission in cocaine dependence. Front Psychiatry 2013;4:44.

7. Tejeda HA, Counotte DS, Oh E, Ramamoorthy S, Schultz-Kuszak KN, Backman CM*, et al*. Prefrontal cortical kappa-opioid receptor modulation of local neurotransmission and conditioned place aversion. Neuropsychopharmacology 2013;38:1770-1779.

8. Du C, Duan Y, Wei W, Cai Y, Chai H, Lv J*, et al*. Kappa opioid receptor activation alleviates experimental autoimmune encephalomyelitis and promotes oligodendrocyte-mediated remyelination. Nat Commun 2016;7:11120.