Quantitative signaling and structure-activity analyses demonstrate functional selectivity at the nociceptin/orphanin FQ opioid receptor.

Steven D. Chang, S. Wayne Mascarella, Skylar M. Spangler, Vsevolod V. Gurevich, Hernan A. Navarro, F. Ivy Carroll, Michael R. Bruchas

Supplemental Information

Figure S1 – BRET Transient Transfection Titration

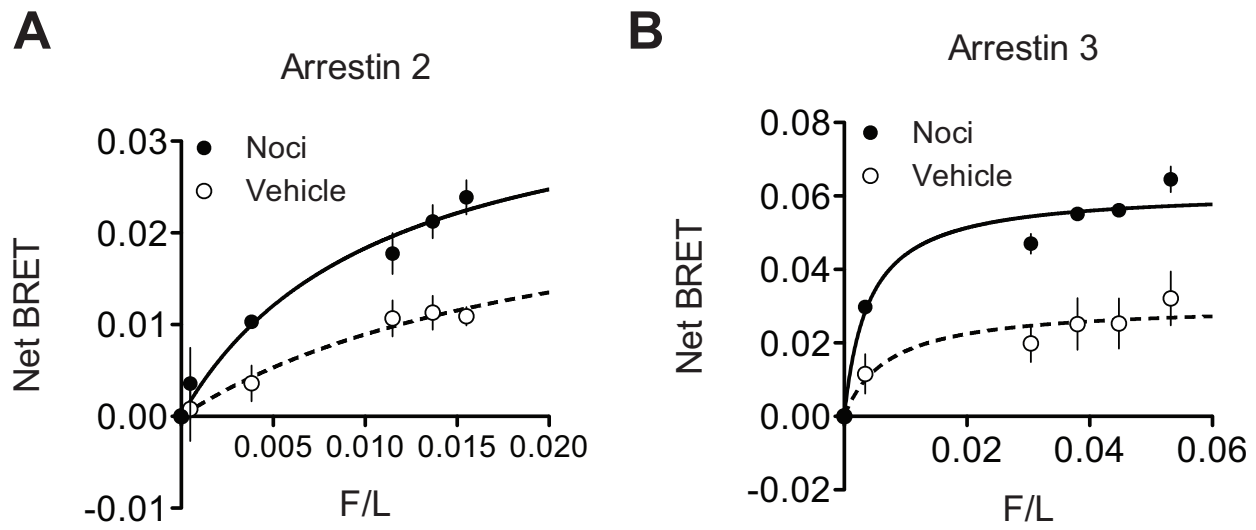

Figure S1. BRET Transient Transfection Titration Curves. An acceptor saturation experiment was used to determine optimal donor-acceptor transfection stoichiometry between NOPR-Rluc8 (BRET donor) and arrestin3-Venus (**A**) or arrestin2-Venus (**B**) as BRET acceptor. NOPR-Rluc8 (125ng) and increasing amounts of acceptor (0, 250, 500, 750, 1000, or 1250ng) were transfected into HEK293 cells. Hyperbolic curves denote a constitutive baseline interaction between NOPR and arrestin2/3 that is increased upon treatment with nociceptin. From this experiment, we used 1000ng of acceptor, yielding the optimal ratio of 1:8, NOPR-Rluc8:arrestin2/3-Venus. Data represent mean ± SEM net BRET between NOPR-arrestin3 interactions and NOPR-arrestin2 after treatment with nociceptin (●) and vehicle control (○) in a representative experiment in triplicate.

Figure S2 – Ligand-induced Arrestin Recruitment

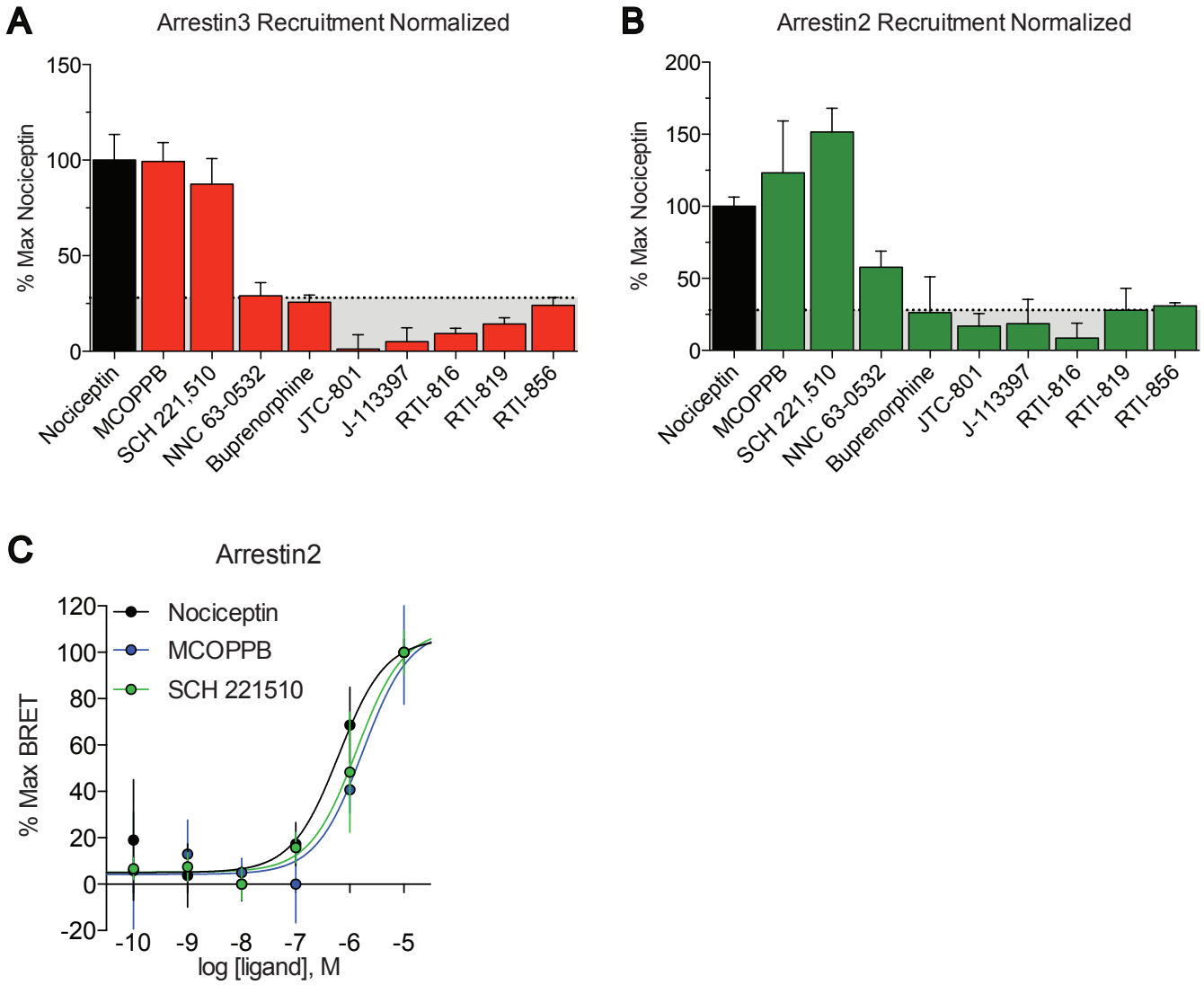

Figure S2. Ligand-induced Arrestin Recruitment. (**A,B**) Normalized maximum NOPR ligand-induced recruitment of arrestin3 (**A**) and arrestin2 (**B**). MCOPPB and SCH 221,510 show full agonist activity and are not significantly different from nociceptin (reference ligand), while all other ligands tested show no significant arrestin recruitment difference from vehicle controls. Ligand induced recruitment of arrestin2 is similar, with the exception of SCH 221,510 which is significantly different from both nociceptin and vehicle control (represented as a dotted line). (**C**) Normalized concentration response curves for arrestin2 recruitment to NOPR showing rank order of potency for tested agonists (n ≥ 3, x3 replicates). Both MCOPPB and SCH 221,150 have shifted EC^{50}. All points are mean ± SEM.

Figures S3 – G-protein and arrestin pathway bias plots

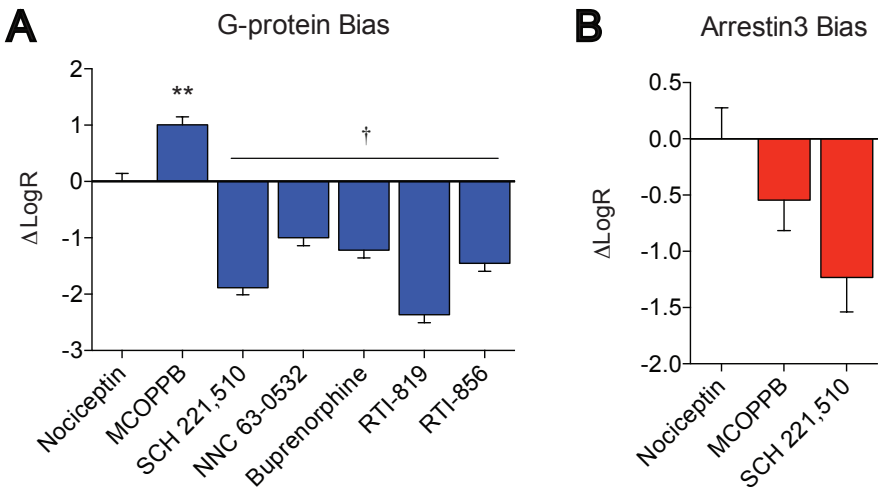

Figure S3. G-protein and arrestin pathway bias plots. (**A,B**) Calculated transduction coefficients (ΔlogR) for ligands in G-protein (**A**) and arrestin3 (**B**) signaling relative to reference ligand, nociceptin. MCOPPB shows a significantly higher bias for G-protein signaling, while all other agonists are significantly lower. SCH 221,510 shows a significantly lower bias for arrestin3 when compared to nociceptin. All points are mean ± SEM (** = positive bias $p \leq 0.01$, † = negative bias $p \leq 0.01$, n = 3-6, triplicate samples).

Table S1 – RTI compound binding affinity data

	NOPR	μ	δ	κ
Compound	**K_e (nM)**	**K_e (nM)**	**K_e (nM)**	**K_e (nM)**
RTI-816	6.86 ± 1.02	200	1120 ± 134	465 ± 62
RTI-819	3.52 ± 0.85	101 ± 13	10,800	317 ± 39
RTI-856	28	ND	2970 ± 300	4000 ± 1500

Table S1. Selectivity data for novel compounds synthesized in this study. All ligands show high selectivity for NOPR over all other opioid receptors (~30-1500 fold). (ND = not determined).

Table S2. Previously published NOPR ligand binding affinity and activity data.

	Binding Affinity	**Activity**	
Ligand	pK_i	$logEC_{50}$ (nM)	**Reference**
Nociceptin	9.7	-8.98	Okawa et al. (1999) Br J Pharmacol.
SCH 221,510	9.52	-7.92	Varty et al. (2008) J Pharmacol Exp Ther.
MCOPPB	10.1	-9.41	Hirao et al. (2008) J Pharmacol Sci.
NNC 63-0532	8.13	-6.52	Thomsen and Hohlweg (2000) Br J Pharmacol.
Buprenorphine	6.54	-7.46	Huang et al. (2001) J Pharmacol Exp Ther.
JTC-801	8.08	-8.59	Shinkai et al. (2000) J. Med. Chem. Yamada et al. (2002) Br J Pharmacol.
J-113,397	8.74	-8.28	Ozaki et al. (2000) Eur J Pharmacol.

Table S2. Published NOPR affinity and $logEC_{50}$ data for NOPR activity. MCOPPB has highest affinity for NOPR, while Buprenorphine is less selective. Published pK_i are from a multitude of cell types. Published activities are also from a variety of assays.