

Multifunctional Opioid Receptor Agonism and Antagonism by a Novel Macrocyclic Tetrapeptide Prevents Reinstatement of Morphine-Seeking Behavior

Ariana C. Brice-Tutt¹, Lisa L. Wilson¹, Shainnel O. Eans¹, Heather M. Stacy¹, Chloe A. Simons¹, Grant Simpson², Jeremy S. Coleman², Michael J. Ferracane²⁺, Jane V. Aldrich² and Jay P. McLaughlin¹

Appendix S1: Specific numbers of mice used across experiments:

Animals were not re-used across different experiments, but time points from the same experiments are displayed across several figures as needed to clarify the characterization.

Use of the animals by experiment is listed in the following table:

Figure

	C57BJ/6			MOPr KO		KOPr KO		DOPr KO		Total per Figure:
	<i>cyclo</i> [Pro-Sar-Phe-D-Phe]	Vehicle	Opioid Controls	<i>cyclo</i> [Pro-Sar-Phe-D-Phe]	Vehicle	<i>cyclo</i> [Pro-Sar-Phe-D-Phe]	Vehicle	<i>cyclo</i> [Pro-Sar-Phe-D-Phe]	Vehicle	
2A	92*	8								100
2B	22	7								29
3	16			8		8		8		40
4A	32		48 [†]							80
4B	24* + 15		16 [†]							55
4C	16*		16 [†]							32
4D	8		16 [†]							24
5	8	32	8							48
6	22	39	16	17	24	16	20			154
7	41	21	47							109
8A	96	94	12							202
8B	21	20	14							55
Total Mice Used	739			49		44		8		840

(blue* = additional data from mice also shown in figure 2A; blue[†] = data from the 16 U50,488 controls shown in figure 4A)

Appendix S2: Morphine antinociceptive dose response:

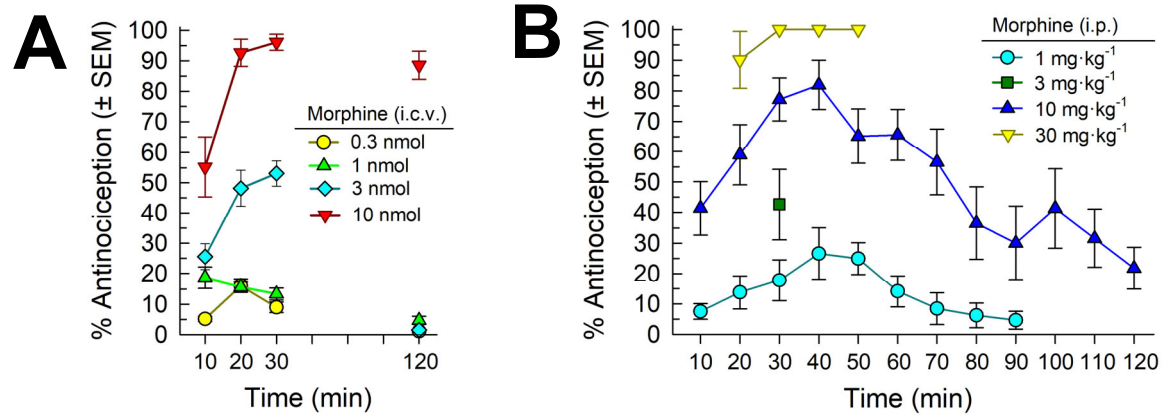


Figure S1. Antinociceptive activity of morphine following administration i.c.v. (**A**) or i.p. (**B**) in the 55°C warm-water tail-withdrawal assay in C57BL/6J mice. Morphine demonstrated significant time- and dose-dependent antinociception with repeated measurement over time. Points represent average % antinociception \pm SEM from 8 mice for each set (16 for 10 mg·kg⁻¹ i.p.) presented for a total of 63 C57BL/6J mice.