β-Arrestin 2 Downregulation on Morphine-Induced Antinociceptive Tolerance in Rodents: A Systematic Review and Meta-Analysis

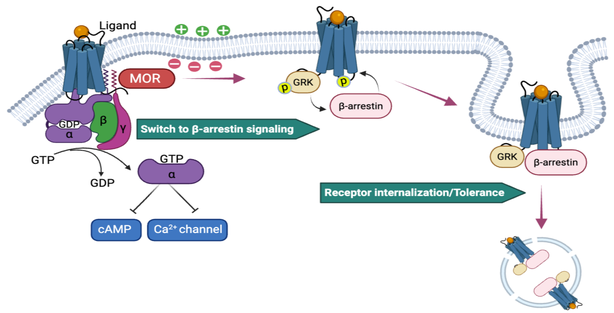
How Rodent Models Help Us Understand Pain Treatment

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INTRODUCTION

- *β-Arrestin 2* (βarr2) is an intracellular protein that regulates GPCR signaling, including the μ-Opioid Receptor (MOR).
- Multiple preclinical studies use diverse molecular strategies to study whether reducing βarr2 activity preserves morphine analgesia over time.
- Strategies include, Genetic Knockout, Antisense Oligonucleotides, Small Interfering RNAs (siRNAs), Antigene RNAs (agRNAs)
  MicroRNAs (miRNAs)
- However, no quantitative synthesis of this evidence has been performed.
- Therefore, We quantitatively synthesize preclinical evidence to answer whether reducing βarr2 activity also reduces morphine antinociceptive tolerance in rodent models.

Role of β-arrestin in Opioid tolerance



METHODS

- A systematic literature search identified rodent studies measuring antinociception of chronic morphine administration
- Studied %MPE (Maximum Possible Effect) on Day 7 of Chronic Morphine Administration in both βarr2-deficient and wild-type/control rodent groups
- Hedges' g was computed for each study as the primary effect size.
- Cochran's Q and I^2 statistics quantified between-study heterogeneity
- Using DerSimonian-Laird method

RESULTS

- In the 6 studies included for data analysis, The pooled Hedges' g was 1.38 (95% CI: 0.94–1.83), indicating a large, statistically significant, effect of βarr2 downregulation.
- Heterogeneity was negligible (I^2 = 0%, τ^2 = 0.000, Q = 1.72, df = 5, p = 0.89), indicating differences between the studies were negligible.

Inhibiting βarr2 activity consistently **reduces morphine tolerance** across various **rodent models,** methods, and brain regions

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Included studies

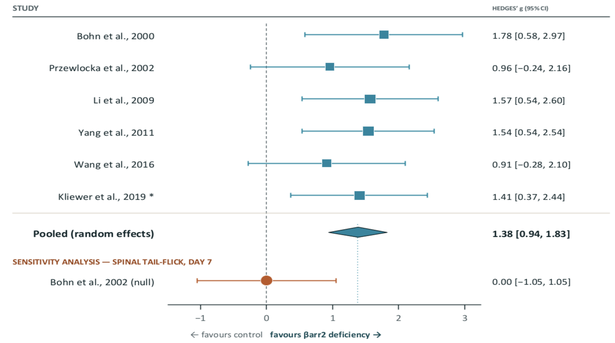
Table 1. Characteristics and effect sizes, Day 7 of chronic morphine.

Study	Species	Intervention	Assay	n c/e	%MPE ctrl	%MPE exp	Hedges' g
Bohn, 2000	Mouse (B6 × 129SvJ)	Global βarr2-KO	Tail-immersion 54°C	7 / 8	21	79	1.78
Przewlocka, 2002	Rat (Wistar)	AS-ODN, i.th.	Tail-flick	6 / 6	13	30	0.96
Li, 2009	Mouse (C57BL/6)	siRNA lentivirus, PAG	Hot-plate 52°C	10 / 9	12	45	1.57
Yang, 2011	Rat (Sprague-Dawley)	siRNA, i.th.	Tail-flick 54°C	10 / 10	15	38	1.54
Wang, 2016	Rat (Sprague-Dawley)	miR-365 lentivirus, i.th.	Tail-flick	6 / 6	0	10	0.91
Kliewer, 2019 *	Mouse (C57BL/6J)	Phospho-deficient MOR KI	Hot-plate 56°C	9 / 9	15	50	1.41
Pooled (random effects) — I^2 = 0%, τ^2 = 0.000, Q = 1.72, df = 5, p = 0.89							**1.38**

AS-ODN = antisense oligonucleotide; i.th. = intrathecal; PAG = periaqueductal gray; KI = knockin; %MPE = percent maximum possible effect. SD derived from reported SEM (SD = SEM × √n).

Pooled effect on Day 7 antinociception

Figure 2. Random-effects forest plot. Square area is proportional to study weight; horizontal bars are 95% CIs; the diamond is the pooled estimate.



* Phosphorylation-deficient MOR knockin (10S/T-A), which functionally prevents βarr2 recruitment. Including Bohn 2002 lowers the pooled estimate to g = 1.14 (95% CI 0.58–1.70); the Bu 2015 von Frey dataset gives g = 1.39 (95% CI 0.13–2.65).

DISCUSSION

A Critical Exception: βarr2 downregulation reliably prevents supraspinal tolerance (hot-plate assay). However, spinal tolerance (tail-flick assay) still develops by Day 7 implicated by a PKCγ mechanism (Bohn et al., 2002).

- Agonist selectivity:
  - βarr2-KO attenuates morphine tolerance (~10-fold smaller ED50 shift at Day 7) but not tolerance to methadone, fentanyl or oxycodone.
- Side effects are not βarr2-driven:
  - Phosphorylation-deficient MOR knockin mice show markedly reduced tolerance yet unchanged or worsened respiratory depression and constipation at equianalgesic doses (ED50 correlation R^2 =0.92–0.97) (Kliewer et al., 2019)
- Overall, more research needs to be conducted to fully understand opioid tolerance mechanisms for more effective pain treatment to prevent dose escalation and adverse opioid side effects.
- References: In Manuscript

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