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Title: Building a Better Analgesic: Multifunctional Compounds that Address Injury-induced Pathology to Enhance Analgesic Efficacy while Eliminating Unwanted Side Effects

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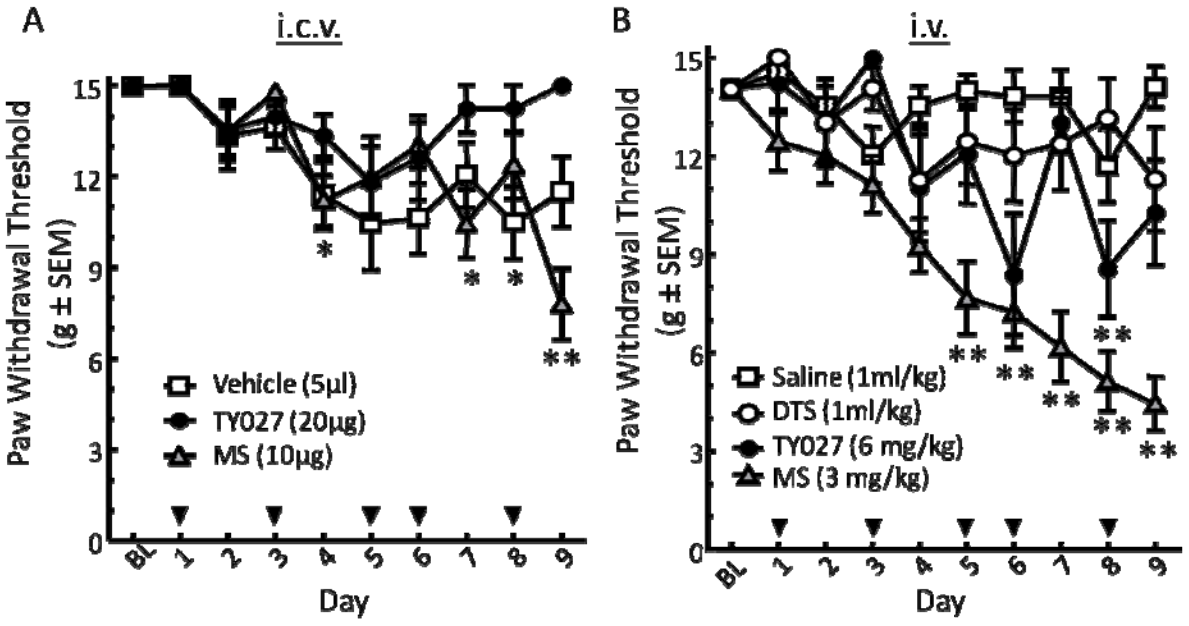
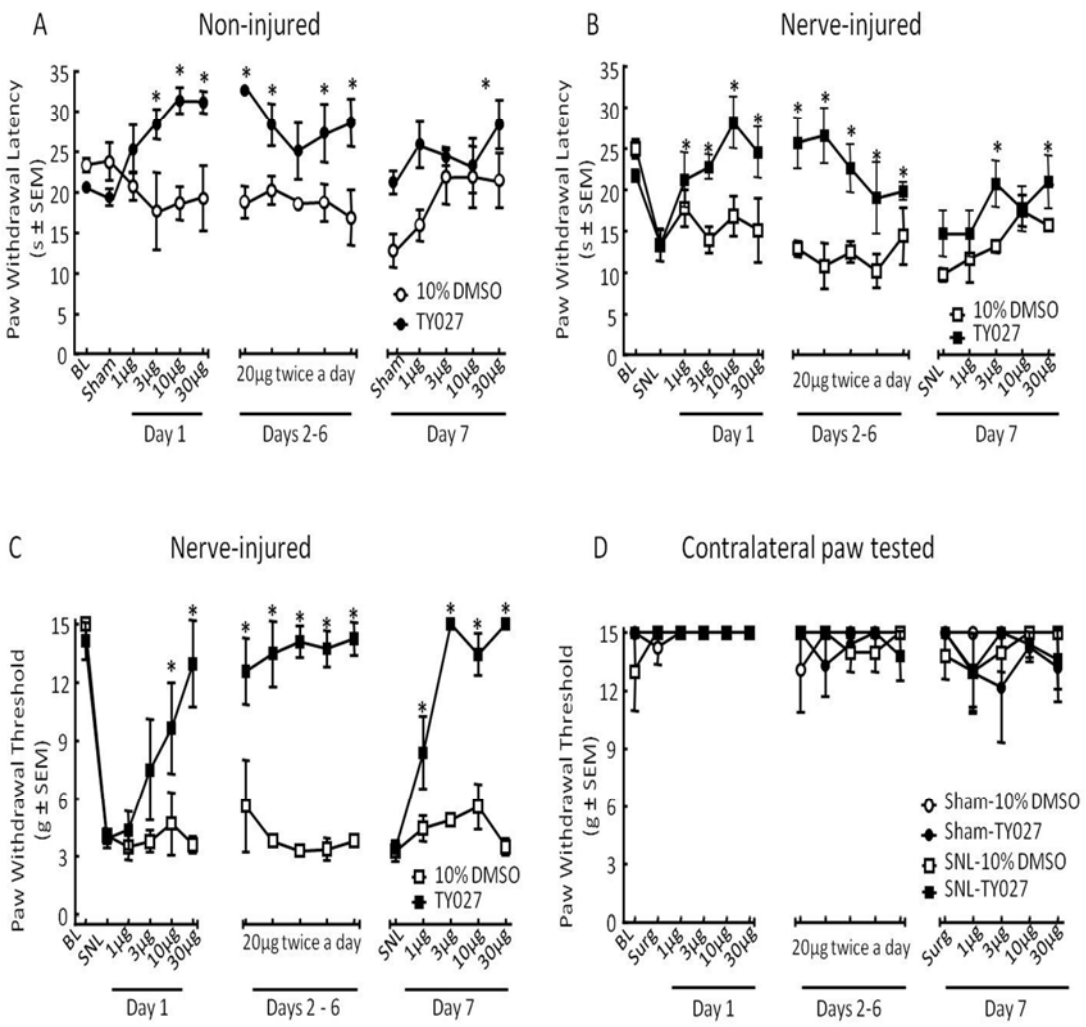
Supplemental Figure 1



Supplemental Figure 1: Paradoxical allodynia develops after supraspinal and systemic administrations of morphine but not TY027. Non-injured rats injected with TY027 (i.c.v. and i.v.) were assessed for the development of compound-induced tactile allodynia 75 - 90 min after exposure/CPP evaluation. (A) Supraspinal (i.c.v.) administration: MS resulted in significant allodynia compared to baseline values beginning after the five exposures (*$p \leq 0.05$). Supraspinal TY027 exposed animals did not show significant decreases in paw withdrawal thresholds ($p = 0.11$) (B) Systemic (i.v.) administration of morphine (MS, 3 mg/kg) induced significant tactile allodynia after the second exposure that continued to decrease until the final assessment on day 9 (*$p \leq 0.05$). TY027-treated rats showed a delayed development of hypersensitivity with the onset occurring on the 5th exposure (**$p \leq 0.01$; day 8); this hypersensitivity resolved in the absence of compound (day 9). Arrowheads indicate day of drug exposure ($n = 8$ - 10 in all experimental groups).

Supplemental Figure 2



Supplemental Figure 2: Lack of tolerance *in vivo* of intrathecal TY027 in SNL and non-injured animals. Spinal TY027 was administered to rats using a cumulative dosing paradigm on days 1 and 7, while 20 µg/5µl was given twice daily on days 2 – 6. Behavioral testing occurred 30 min after injections. (A) Compared to vehicle treated animals and baseline values (day 1), TY027 retained antinociceptive activity over the 7 day time course in sham-operated (non-injured) rats with reconstruction of the dose response curve on day 7. (B) In SNL-operated rats, TY027 significantly reversed thermal hyperalgesia 30 min after spinal administration on day 1 and after seven days of chronic treatment (day 7). (C) Mechanical allodynia induced by SNL was dose-dependently attenuated following TY027 (i.t.) on day one and as

well as on day 7 after repeated administration (day 7). (D) Contralateral PWTs were not significantly changed from baseline with prolonged treatment of vehicle or TY027 in sham- or SNL operated rats. *$p \leq 0.05$. Data represent mean ± SEM, n = 6 - 8 rats/treatment.

Supplemental Table 1: *In vitro* comparison of TY027 and TY005

Compound ID	Affinity				GTPγS binding				MVD	GPI/LMMP			Plasma Half life ($T_{1/2}$)
	hDOR (K_i; nM)	rMOR (K_i; nM)	hNK_1 (K_i; nM)	rNK_1 (K_i; nM)	hDOR		rMOR		Opioid (δ)	Opioid (μ)	Substance P		
					EC_{50} (nM)	Emax (%)	EC_{50} (nM)	Emax (%)	Agonist (IC_{50}; nM)	Agonist (IC_{50}; nM)	Agonist (IC_{50}; nM)	Antagonist (K_e; nM)	
TY005	2.8	36.3	0.082	0.29	2.9	47.6	31.6	45.6	22.3	358.8	None	24.7	0.83 min
TY027	0.66	15.7	0.0064	7.27	8.6	58.0	7.0	55.0	14.5	487.9	None	10	4.83 h

Binding affinities and *in vitro* activities for both compounds, as well as the plasma half-life ($T_{1/2}$) for TY027, are published (Yamamoto et al., 2007; 2009). To determine $T_{1/2}$, peptides were incubated in rat plasma at 37 °C. Aliquots were withdrawn at multiple time points then analyzed by HPLC to determine the concentration of remaining peptide.