SUPPLEMENTAL DATA

MOLECULAR PHARMACOLOGY

R-type ($Ca_v2.3$) Calcium Channel Inhibition via Human μ- δ- and κ-Opioid Receptors is Voltage-Independently Mediated by Gβγ Protein Subunits

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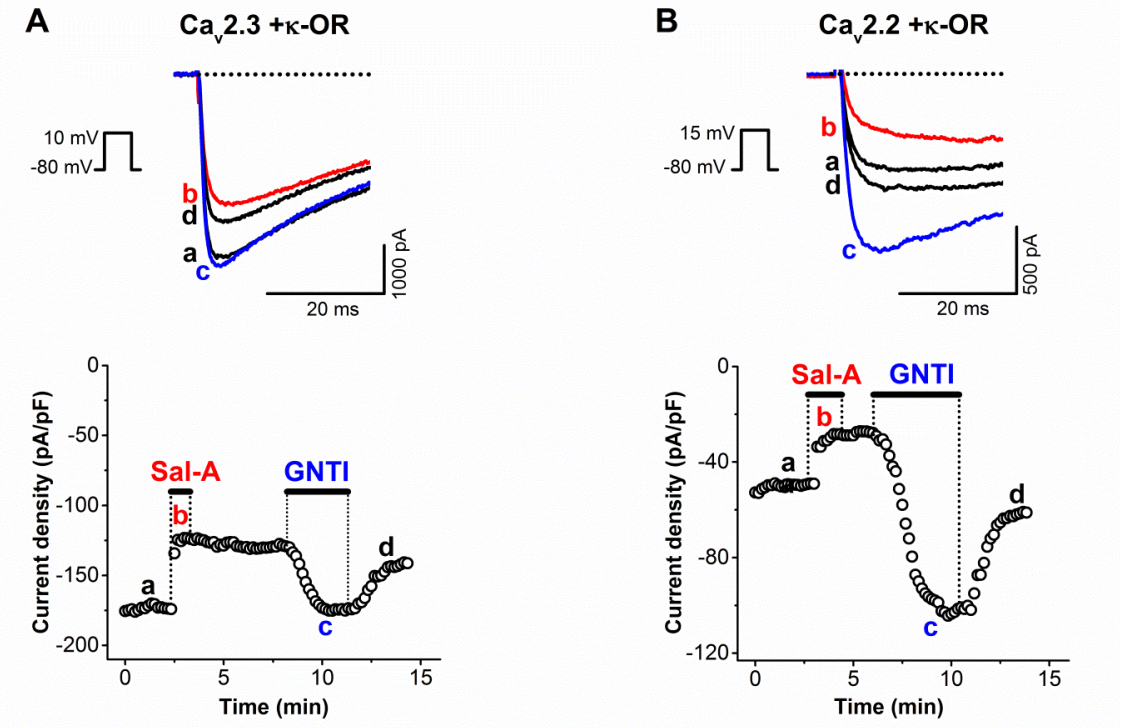
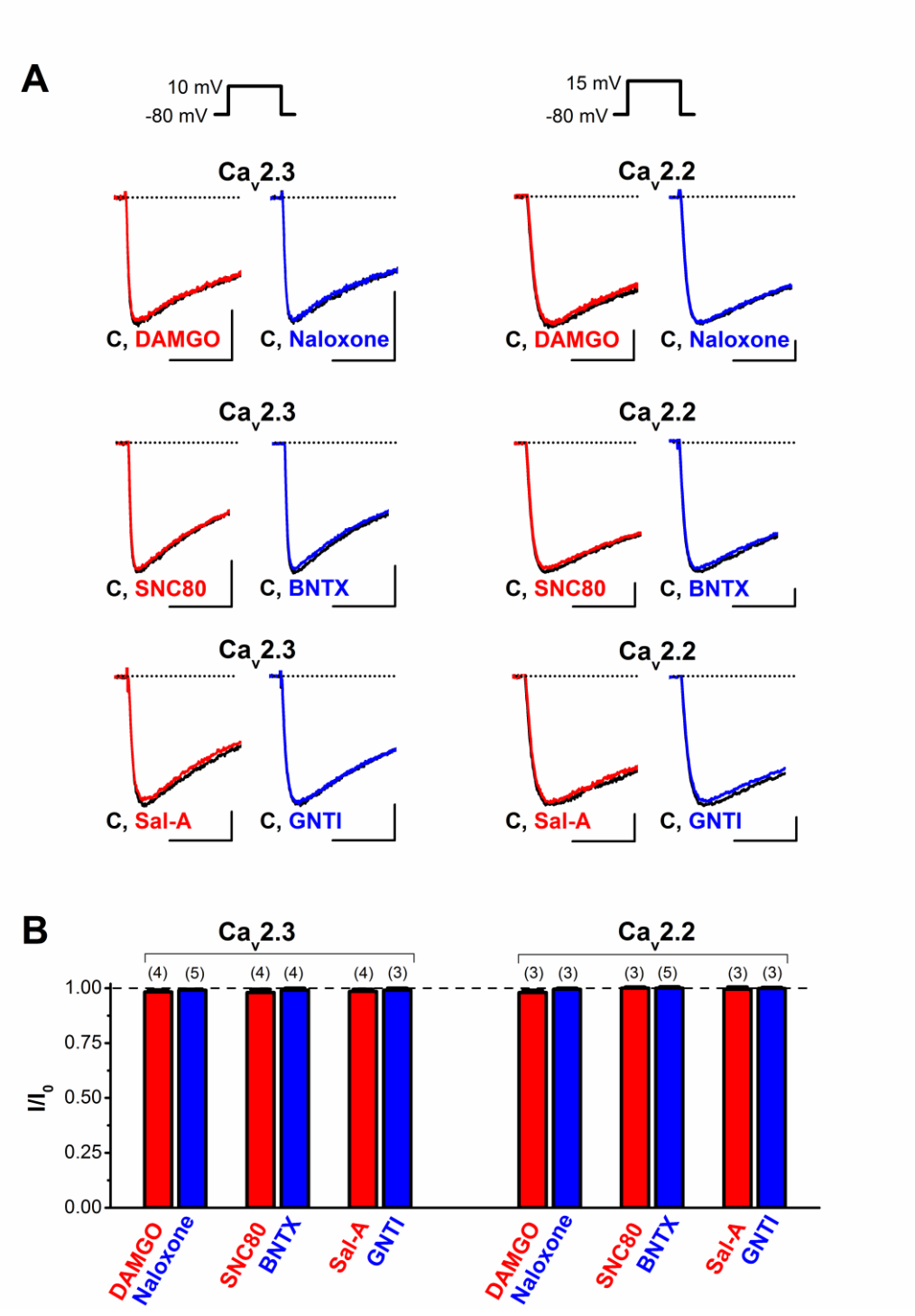
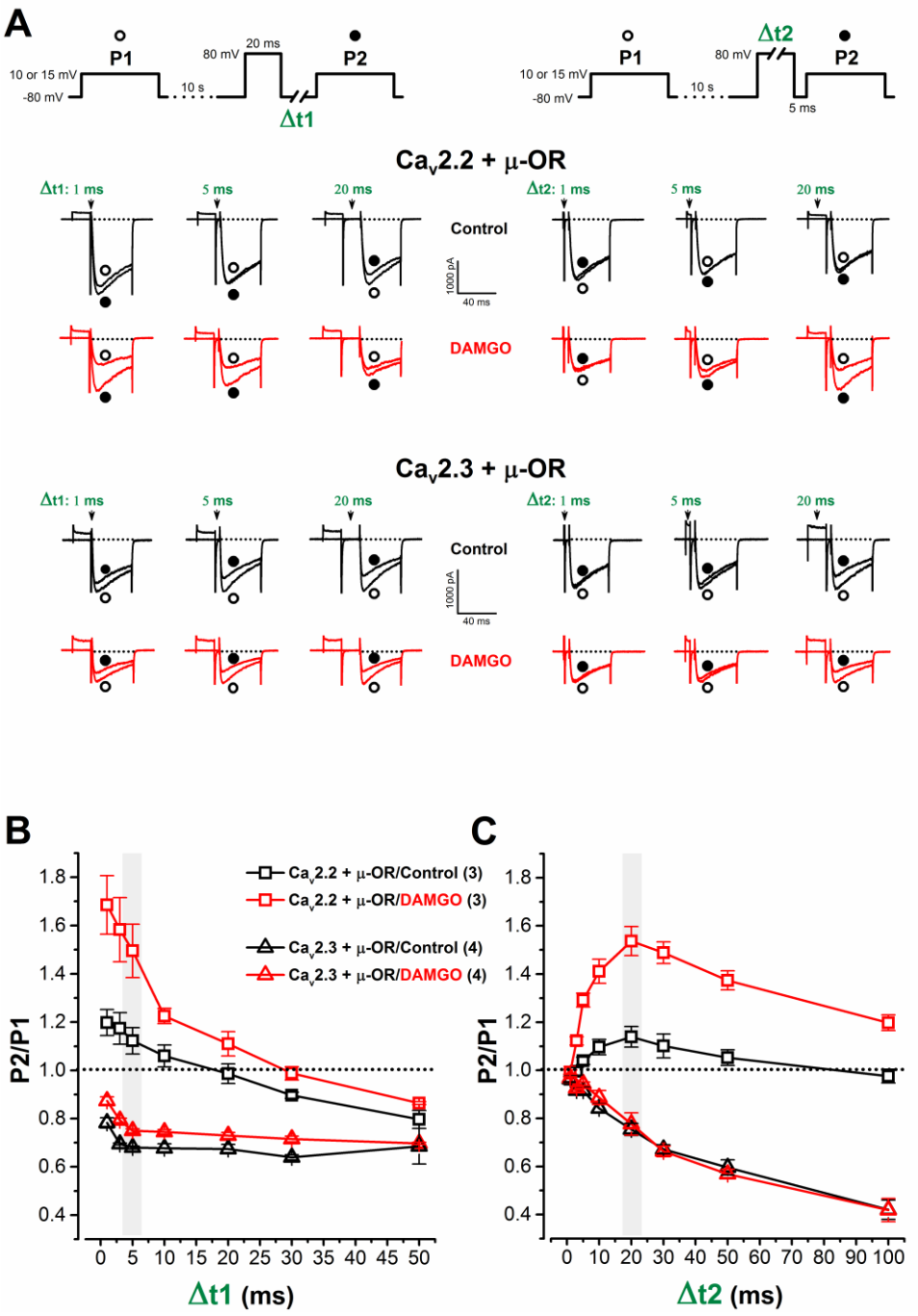


Supplemental Figure 1. Modulation of $Ca_v2.3$ and $Ca_v2.2$ channels by the κ-opioid receptor (OR) agonist Sal-A and the κ-OR antagonist GNTI in HEK cells stably expressing $Ca_v2.3$ or $Ca_v2.2$ channels and transiently co-expressing κ-ORs ($Ca_v2.3$/κ-OR and $Ca_v2.2$/κ-OR cells, respectively). *Bottom:* Time course of peak Ba^{2+} current (I_{Ba}) densities in the presence of Sal-A (1 μM) and subsequently applied GNTI (100 nM) in $Ca_v2.3$/κ-OR (A) and $Ca_v2.2$/κ-OR (B) cells. In both cases, Sal-A inhibiton of I_{Ba} was largely irreversible, exhibiting 7.2 ± 0.6% (n = 17) or 14.8 ± 1.6% (n = 8) recovery after ~5 min washout for $Ca_v2.3$ or $Ca_v2.2$ channels, respectively, whereas the subsequent GNTI potentiation of I_{Ba} was reversible. Bars indicate the duration of Sal-A or GNTI application. *Top:* Representative current traces, shown at the time points indicated by lowercase letters (only 25 ms of the 150-ms traces are shown); dashed lines indicate zero-current level. Peak I_{Ba} amplitudes were evoked by 150 ms depolarizations to +10 mV (A) or +15 mV (B) at 0.1 Hz. This experiment was repeated four and five times for $Ca_v2.3$/κ-OR and $Ca_v2.2$/κ-OR cells, respectively, with similar results.



Supplemental Figure 2. Opioid receptor agonists and antagonists do not modulate I_{Ba} in HEK293 cells stably expressing $Ca_v2.3$ or $Ca_v2.2$ channels alone. A, The effects of μ-, δ-, or κ-OR agonists DAMGO (1 μM), SNC80 (1 μM), and Sal-A (1 μM), respectively, and μ-, δ-, or κ-OR antagonists naloxone (1 μM), BNTX (100 nM), and GNTI (100 nM), respectively, were tested in separate HEK293 cells stably expressing $Ca_v2.3$ or $Ca_v2.2$ channels. Representative whole-cell I_{Ba} traces in the absence (C, control, black) and presence of OR agonists (red) or antagonists (blue); dashed lines indicate zero-current level. Peak I_{Ba} amplitudes were evoked by depolarizations to +10 mV ($Ca_v2.3$) or +15 mV($Ca_v2.2$) at 0.1 Hz (only 50 ms of the 110 ms traces are shown). Vertical bars represent 500 pA; horizontal bars represent 30 ms. B, Summary of experiments shown in A. Data are mean ± SEM; the number of experiments is in parentheses.



Supplemental Figure 3. Determination of the extent of pre-pulse facilitation in HEK cells stably expressing $Ca_v2.2$ or $Ca_v2.3$ channels and transiently co-expressing μ-ORs ($Ca_v2.2$/μ-OR and $Ca_v2.3$/μ-OR cells, respectively). A, In $Ca_v2.2$/μ-OR and $Ca_v2.2$/μ-OR cells, representative I_{Ba} traces were elicited by step depolarizations to +10 and +15 mV, respectively, (P1), in the absence (control) and presence of DAMGO (1 μM). A second depolarizing step (P2) was applied immediately subsequent to a strong depolarizing pre-pulse to +80 mV (*top insets*: voltage protocols). A progressive increase in duration between the pre-pulse and P2 (Δt1 = 1, 3, 5, 10, 20, 30, and 50 ms), while maintaining a pre-pulse duration of 20 ms, revealed the presence and absence of voltage-dependent re-inhibition by Gβγ subunit in $Ca_v2.2$ and $Ca_v2.3$ channels, respectively. Varying the pre-pulse duration (Δt2 = 1, 3, 5, 10, 20, 30, 50, and 100 ms), while maintaining a duration of 5 ms between the pre-pulse and P2, served to estimate the dependence of facilitation on the duration of the depolarizing pre-pulse and/or recovery from Gβγ inhibition. Note the absence of facilitation in $Ca_v2.3$/μ-OR cells. Dotted lines indicate zero-current level. Data in B and C represent average P2/P1 values (± SEM); the numbers of experiments are in parentheses. The shaded regions highlight the Δt1 and Δt2 values used also in experiments described in the Results section.

Supplemental Table 1. Average values of Ba^{2+} current (I_{Ba}) facilitation expressed as the P2/P1 ratio.

Cell type	P2/P1		
	control	agonist	antagonist
$Ca_v2.3$ + μ-OR	0.77 ± 0.02 (7)	^{NS}0.84 ± 0.03 (7)	^{NS}0.73 ± 0.05 (3)
$Ca_v2.3$ + δ-OR	0.77 ± 0.04 (5)	^{NS}0.80 ± 0.03 (5)	^{NS}0.76 ± 0.07 (3)
$Ca_v2.3$ + κ-OR	0.82 ± 0.04 (7)	^{NS}00.85 ± 0.03 (7)	^{NS}00.79 ± 0.03 (5)
$^{tr}Ca_v2.3$ + μ-OR	0.93 ± 0.04 (6)	^{NS}1.01 ± 0.04 (6)	ND
$^{tr}Ca_v2.3$ + δ-OR	0.92 ± 0.04 (4)	^{NS}0.97 ± 0.04 (4)	ND
^{tr}Cav2.3 + κ-OR	0.95 ± 0.02 (5)	^{NS}1.01 ± 0.02 (5)	ND
$Ca_v2.3$ + μ-OR + mPhos	0.72 ± 0.02 (12)	^{NS}0.73 ± 0.05 (5)	^{NS}0.73 ± 0.04 (3)
$Ca_v2.3$ + δ-OR + mPhos	0.74 ± 0.03 (11)	^{NS}0.73 ± 0.04 (5)	^{NS}0.74 ± 0.03 (4)
$Ca_v2.3$ + κ-OR + mPhos	0.77 ± 0.02 (8)	^{NS}0.74 ± 0.05 (5)	ND
$Ca_v2.2$ + μ-OR	1.16 ± 0.02 (9)	^{a}2.4 ± 0.04 (8)	ND
$Ca_v2.2$ + δ-OR	1.23 ± 0.03 (7)	^{b}2.7 ± 0.05 (6)	^{c,d}0.96 ± 0.06 (5)
$Ca_v2.2$ + κ-OR	2.2 ± 0.06 (5)	^{NS}2.1 ± 0.07 (5)	^{e,f}0.99 ± 0.04 (6)
$Ca_v2.2$ + μ-OR + mPhos	0.90 ± 0.03 (9)	^{NS}0.92 ± 0.04 (9)	ND
$Ca_v2.2$ + δ-OR + mPhos	0.90 ± 0.03 (7)	^{NS}0.92 ± 0.01 (4)	ND0.86 (2)
$Ca_v2.2$ + κ-OR + mPhos	0.94 ± 0.02 (9)	^{NS}0.95 ± 0.01 (5)	^{NS}0.92 ± 0.03 (4)

Voltage-dependent relief of inhibition was estimated from the P2/P1 ratio, where P1 and P2 represent peak I_{Ba} amplitude in the absence and presence of a depolarizing pre-pulse, respectively (see Materials and Methods). Cell types: HEK293 stably expressing $Ca_v2.2$ (α_{1B-1}), or $Ca_v2.3$ (α_{1E-3}) channels including $\alpha_{2b}\delta$-1 and $Ca_v\beta_{3a}$ subunits and transiently co-expressing μ-, δ- or κ-opioid receptors ($Ca_v2.2$/μ-, δ-, or κ-OR cells and $Ca_v2.3$/μ-, δ-, or κ-OR cells, respectively); HEK293T transiently co-expressing $Ca_v2.3$ (α_{1E-3}), $\alpha_{2b}\delta$-1, and $Ca_v\beta_2$ subunits and μ-, δ-, or κ-ORs ($^{tr}Ca_v2.3$/μ-, δ-, or κ-OR cells); $Ca_v2.3$/μ-, δ-, or κ-OR or $Ca_v2.2$/μ-, δ-, or κ-OR, transiently co-expressing m-Phos. Values represent mean ± SEM; n, number of experiments in parentheses. DAMGO (1 μM), SNC80 (1 μM), and Sal-A (1 μM) were used as agonists, whereas naloxone (1 μM), BNTX (100 nM), and GNTI (1 00 nM) were used as antagonists of μ-, δ-, or κ-ORs, respectively. Student's *t*-test for two groups, $^{a}P < 0.002$ *versus* $Ca_v2.2$/μ-OR/control, and one-way ANOVA with Bonferroni post-hoc testing, $^{b}P < 0.001$, $^{c}P = 0.003$ *versus* $Ca_v2.2$/δ-OR/control, $^{d}P < 0.001$, *versus* $Ca_v2.2$/δ-OR/agonist, $^{e}P < 0.001$ *versus* $Ca_v2.2$/κ-OR/control, $^{f}P < 0.001$, *versus* $Ca_v2.2$/κ-OR/agonist, were used to test for statistically significant differences. NS, not significantly different from the corresponding control; ND, not determined.