SUPPLEMENTAL INFORMATION:

Title: Bias Analyses of Preclinical and Clinical D2 Dopamine Ligands: Studies with Immediate and Complex Signaling Pathways

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Supplemental Table 1:

Compound	Log *K*i
Dopamine	-4.83 (±0.04)
Quinpirole	-5.03 (±0.09)
Aripiprazole	-7.40 (±0.08)
Lisuride	-9.27 (±0.17)
Bromocriptine	-8.02 (±0.10)
Rotigotine	-7.10 (±0.06)
(+)-3-PPP	-4.61 (±0.05)
RNPA	-7.89 (±0.04)
Pramipexole	-5.53 (±0.08)
Ropinirole	-5.28 (±0.14)
Pergolide	-6.49 (±0.15)

Table S1: Affinity constants of the compounds for the D_2 receptor. Log *K*i values and standard errors are shown in the table. The results are an average and S.E.M. of at least three independent experiments.

Supplemental Figure S1:

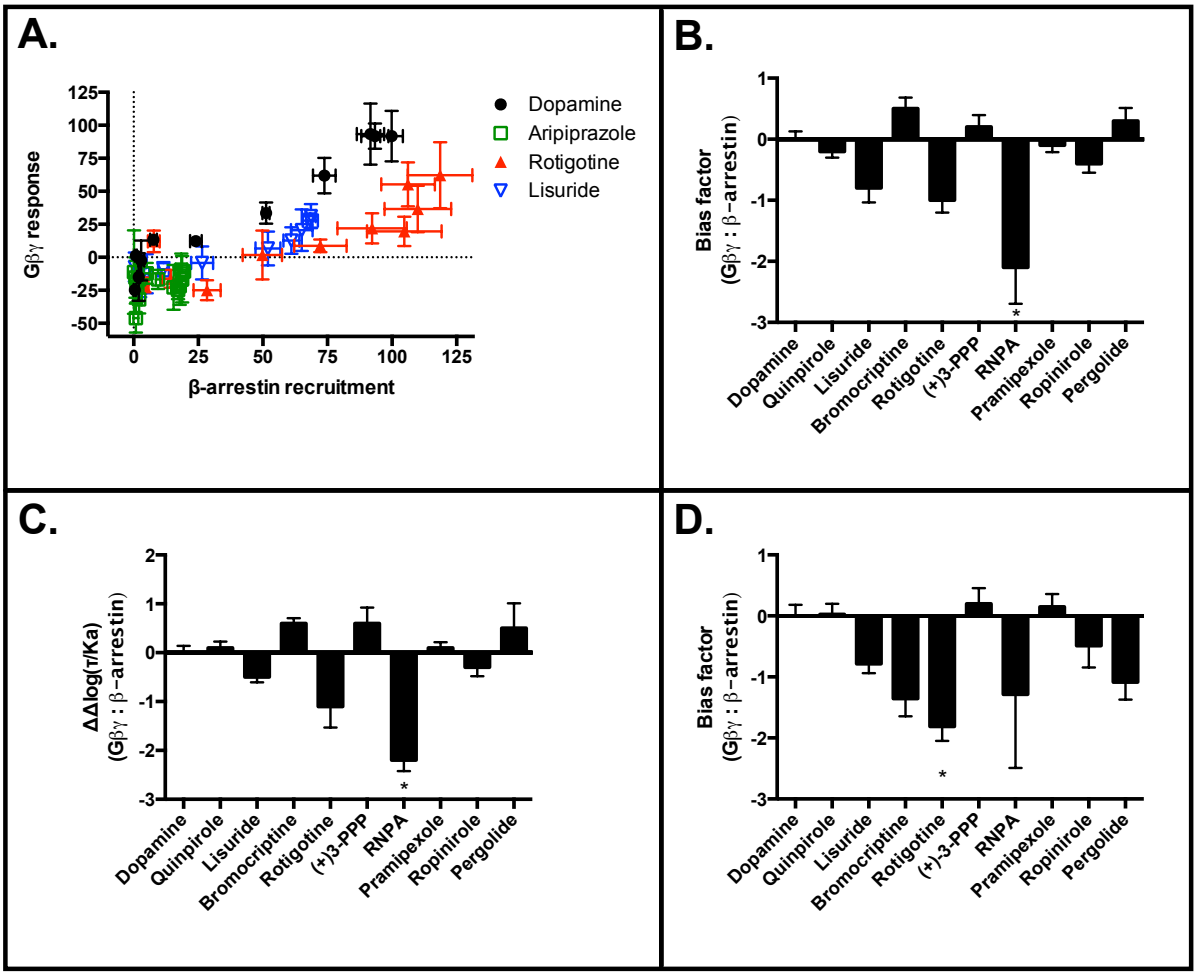

Figure S1: Bias analyses of Gβγ activation in comparison to β-arrestin recruitment to the D_2 receptor. **A.** Equimolar comparison. **B.** Equiactive comparison. **C.** Transduction coefficient. **D.** Sigma comparison. Dopamine was used as the reference compound for all the analyses. For the quantitative analyses positive values indicate bias for Gβγ activation; negative values indicate bias for β-arrestin recruitment. Data represent the average and S.E.M. of at least three independent experiments. *$p < 0.05$.

Supplemental Figure S2:

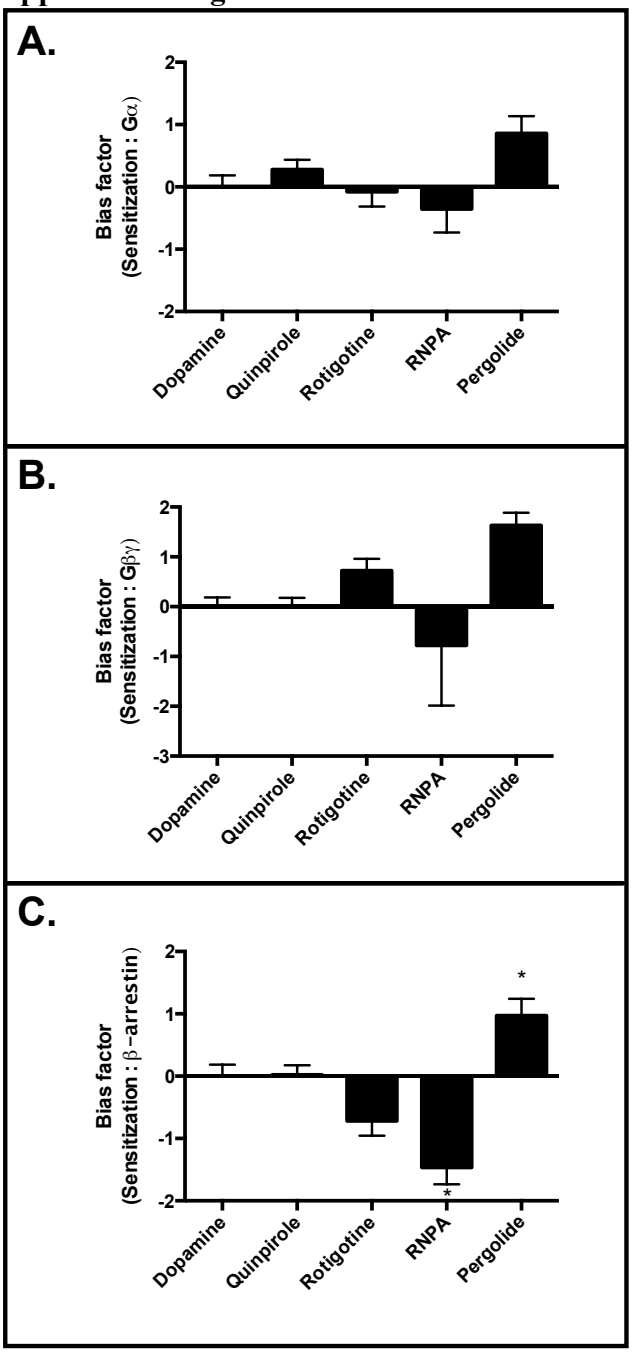

Figure S2: Bias analyses using the sigma comparison of heterologous sensitization in comparison to effectors of the D_2 receptor. **A.** Heterologous sensitization in comparison to Gαi/o activation. **B.** Heterologous sensitization in comparison to Gβγ activation. **C.** Heterologous sensitization in comparison to β-arrestin recruitment. Dopamine was used as the reference compound for all the analyses. Positive values indicate bias for heterologous sensitization; negative values indicate bias for the immediate D_2 receptor effector under analysis. Data represent the average and S.E.M. of at least three independent experiments. *$p < 0.05$.

Supplemental Figure S3:

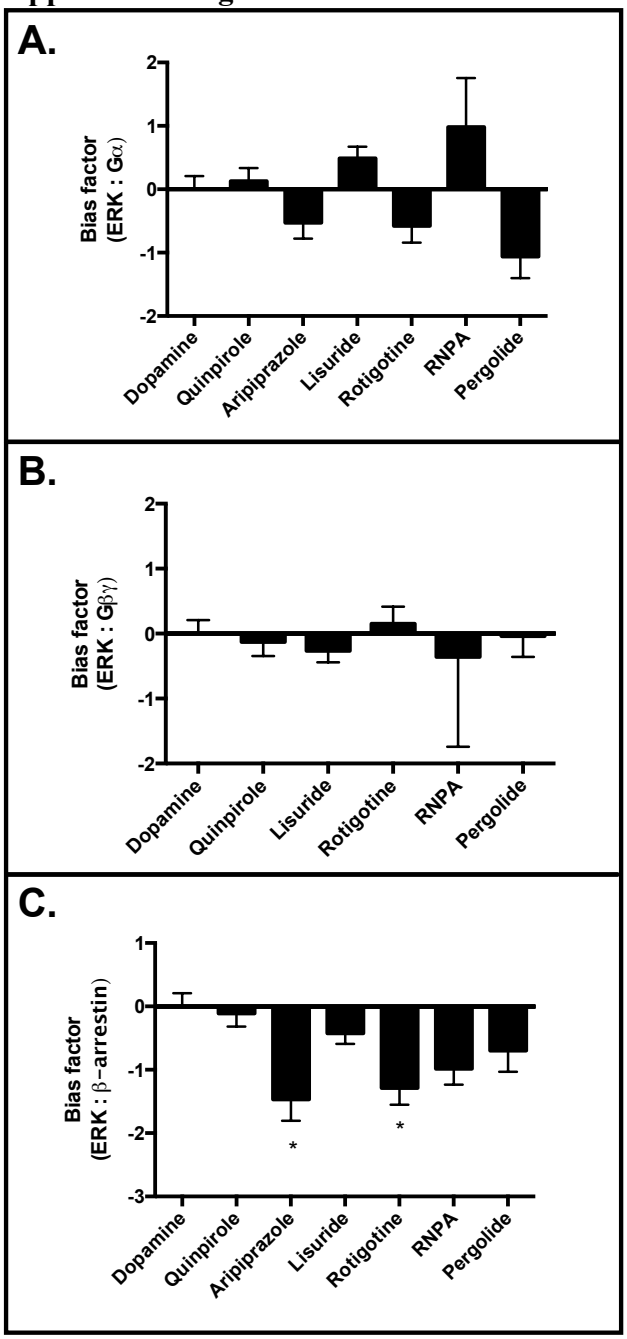

Figure S3: Bias analyses using the sigma comparison of ERK phosphorylation in comparison to effectors of the D_2 receptor. **A.** ERK phosphorylation in comparison to Gαi/o activation. **B.** ERK phosphorylation in comparison to Gβγ activation. **C.** ERK phosphorylation in comparison to β-arrestin recruitment. Dopamine was used as the reference compound for all the analyses. Positive values indicate bias for ERK phosphorylation; negative values indicate bias for the immediate D_2 receptor effector under analysis. Data are the average and S.E.M. of at least three independent experiments. *$p < 0.05$.

Supplemental Figure S4:

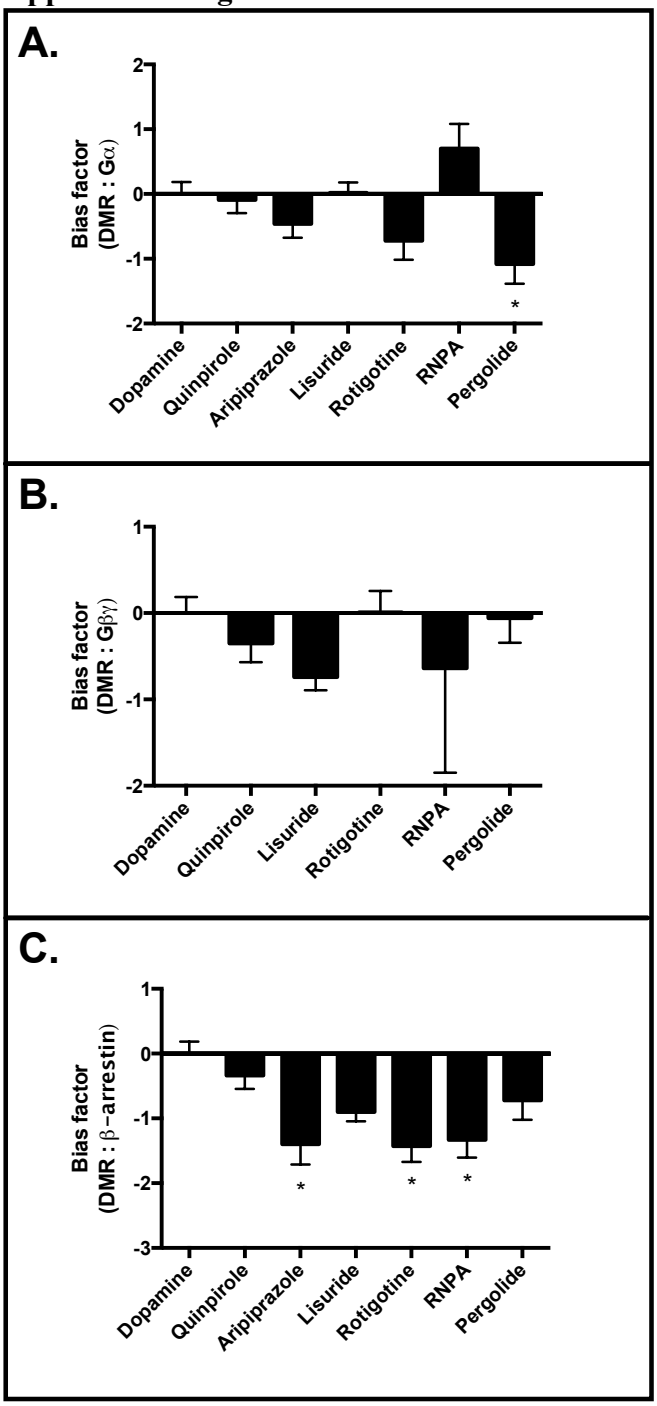

Figure S4: Bias analyses using the sigma comparison of DMR in comparison to effectors of the D_2 receptor. **A.** DMR in comparison to Gαi/o activation. **B.** DMR in comparison to Gβγ activation. **C.** DMR in comparison to β-arrestin recruitment. Dopamine was used as the reference compound for all the analyses. Positive values indicate bias for DMR; negative values indicate bias for the immediate D_2 receptor effector under analysis. Data are the average and S.E.M. of at least three independent experiments. *$p < 0.05$.

Supplemental Figure S5:

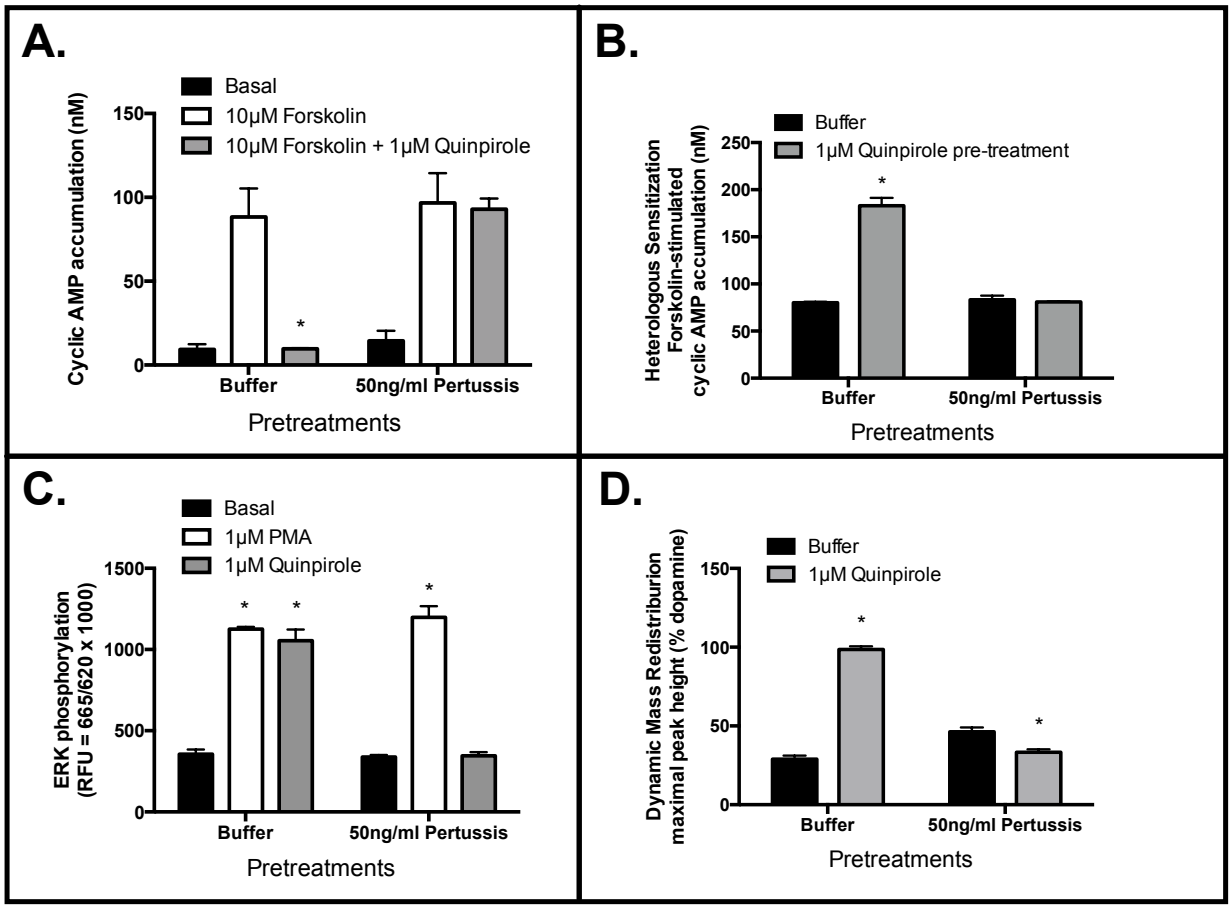

Figure S5: Effects of pertussis toxin pretreatment on Gαi/o activation, heterologous sensitization, ERK phosphorylation, and DMR. Cells were treated overnight (16-18 h) with 50 ng/ml before the functional assays were initiated. Pertussis treatment inhibited quinpirole-mediated inhibition of cAMP production (**A.**), heterologous sensitization (**B.**), D_2 receptor-mediated ERK phosphorylation (**C.**), quinpirole-induced DMR changes (**D.**). Data are the average and S.E.M. of at least three independent experiments. *$p < 0.05$ (*t* test with Holm-Sidak method comparing 10 μM forskolin with 10 μM forskolin + 1 μM quinpirole [**A.**], buffer with 1 μM quinpirole [**B.** and **D.**], and basal with 1μM PMA or 1 μM quinpirole [**C.**].