



Supplementary Information for

Cofactor-enabled functional expression of fruit fly, honeybee, and bumblebee nicotinic receptors reveals picomolar neonicotinoid actions

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Figures S1 to S7

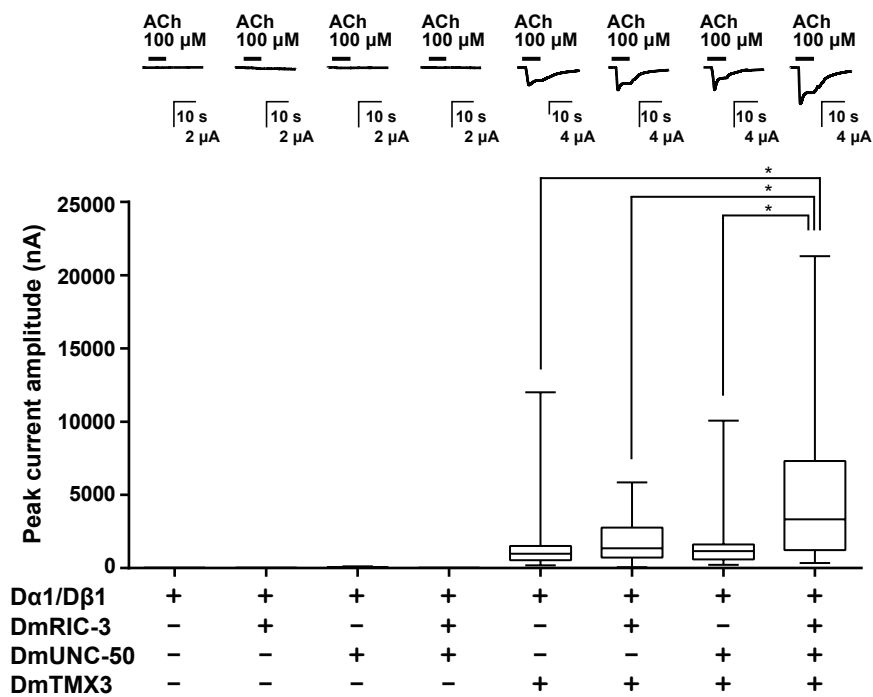


Fig. S1. Effects of cofactors on functional expression of *D. melanogaster* Dα1/Dβ1 nAChRs in *X. laevis* oocytes. Response to 100 μM ACh of *X. laevis* oocytes injected with cRNAs encoding the *D. melanogaster* Dα1/Dβ1 nAChR subunits in combination with cRNAs encoding auxiliary proteins are shown above the Box-whisker plots. In each current record, a horizontal bar indicates bath application of 100 μM ACh. Box plots extend from the 25th to the 75th percentile of the data and the whiskers go down to the minimal and up to the maximal data. The horizontal line in each box indicates the median (n = 47, 4 frogs). *, P < 0.05 (one-way ANOVA, Kruskal-Wallis test).

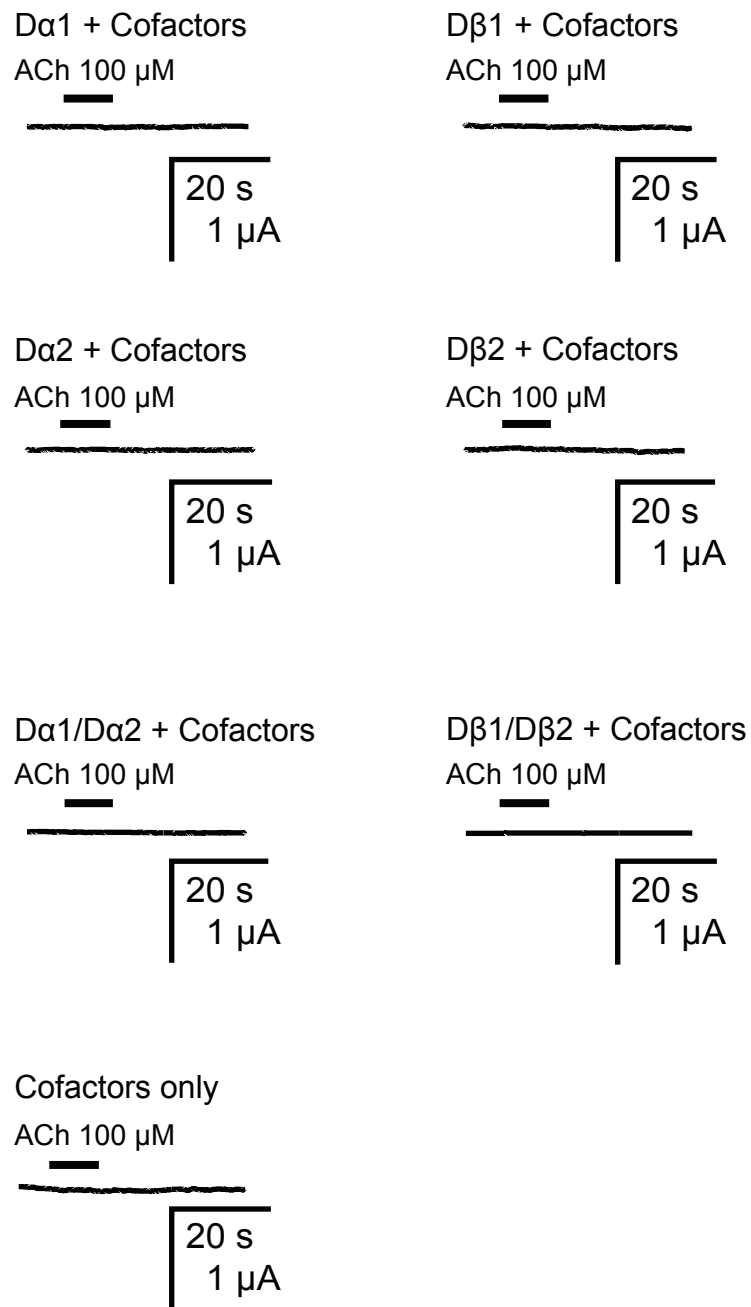


Fig. S2. Responses to ACh of *X. laevis* oocytes expressing *D. melanogaster* nAChR subunits Dα1, Dα2, Dβ1 or Dβ2 alone, the Dα1/Dα2 pairing and the Dβ1/Dβ2 pairing in combination with three cofactors (DmRIC-3, DmUNC-50, and DmTMX3). Horizontal bars indicate application of ACh. Reproducibility of the results were confirmed by repeated experiments (n = 10, 2 frogs).

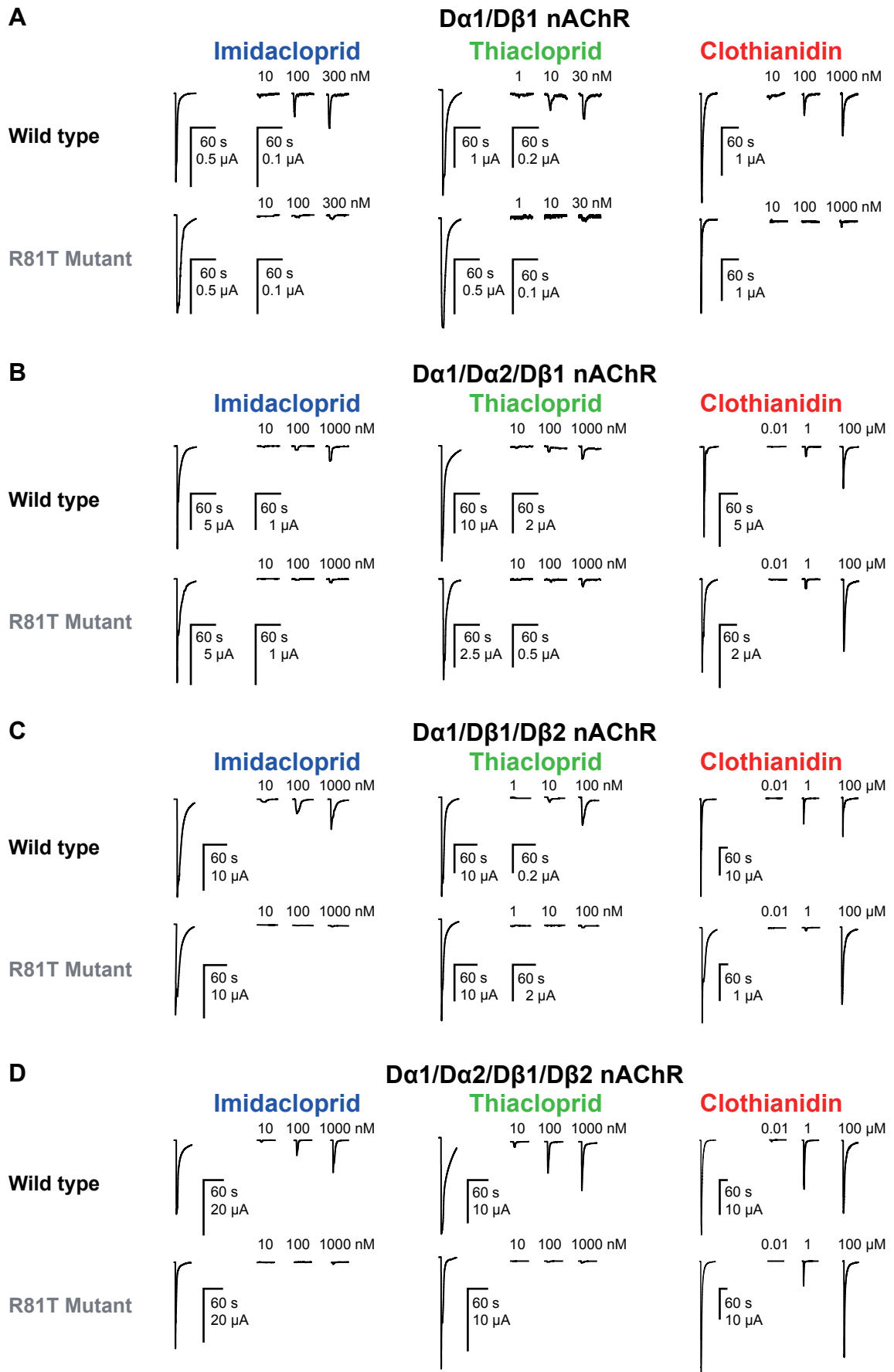


Fig. S3. Responses to acetylcholine and neonicotinoids of wild type and R81T mutant *D. melanogaster* nAChRs. (A) $\text{D}\alpha 1/\text{D}\beta 1$ nAChR, (B) $\text{D}\alpha 1/\text{D}\alpha 2/\text{D}\beta 1$ nAChR, (C) $\text{D}\alpha 1/\text{D}\beta 1/\text{D}\beta 2$ nAChR, and (D) $\text{D}\alpha 1/\text{D}\alpha 2/\text{D}\beta 1/\text{D}\beta 2$ nAChRs. Responses of the wild type and R81T mutant nAChRs for each subunit combination are compared.

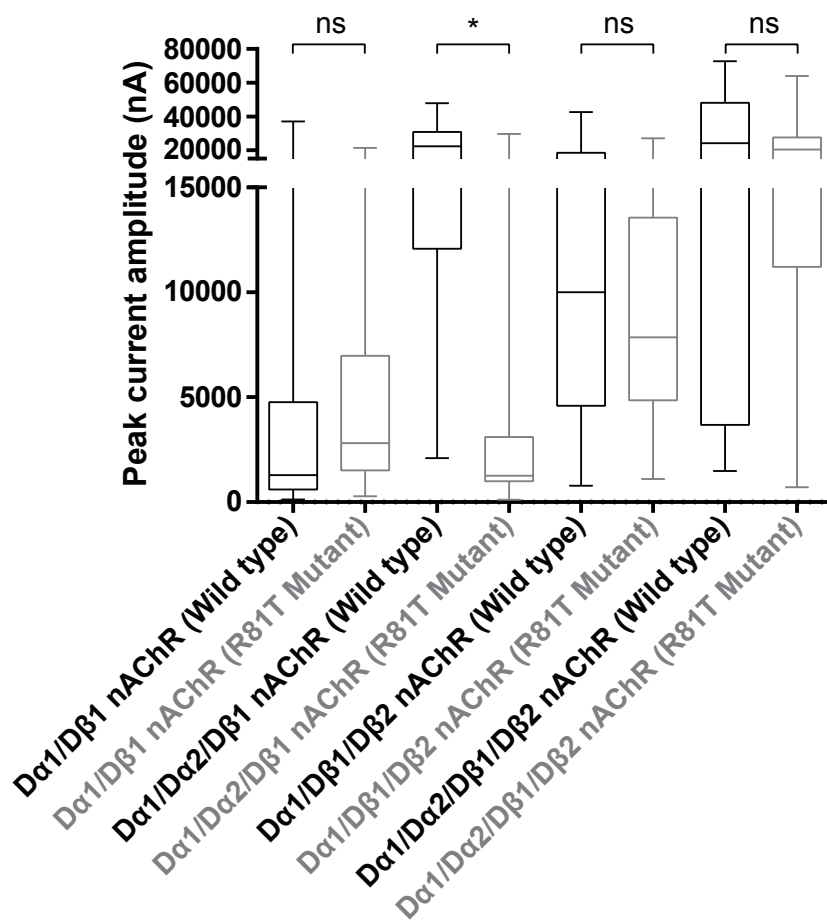


Fig. S4. Peak current amplitude of responses to 100 μ M ACh of wild type and R81T mutant *D. melanogaster* nAChRs (Da1/Dβ1, Da1/Da2/Dβ1, Da1/Dβ1/Dβ2, and Da1/Da2/Dβ1/Dβ2) expressed in combination with DmRIC-3, DmUNC-50, and DmTMX3 in *X. laevis* oocytes. Box plots extend from the 25th to the 75th percentile of the data and the whiskers go down to the minimal and up to the maximal data. The horizontal line in each box indicates the median (n = 45, ≥ 2 frogs). ns, not significant. *, $P < 0.05$ (One-way ANOVA, Kruskal-Wallis test).

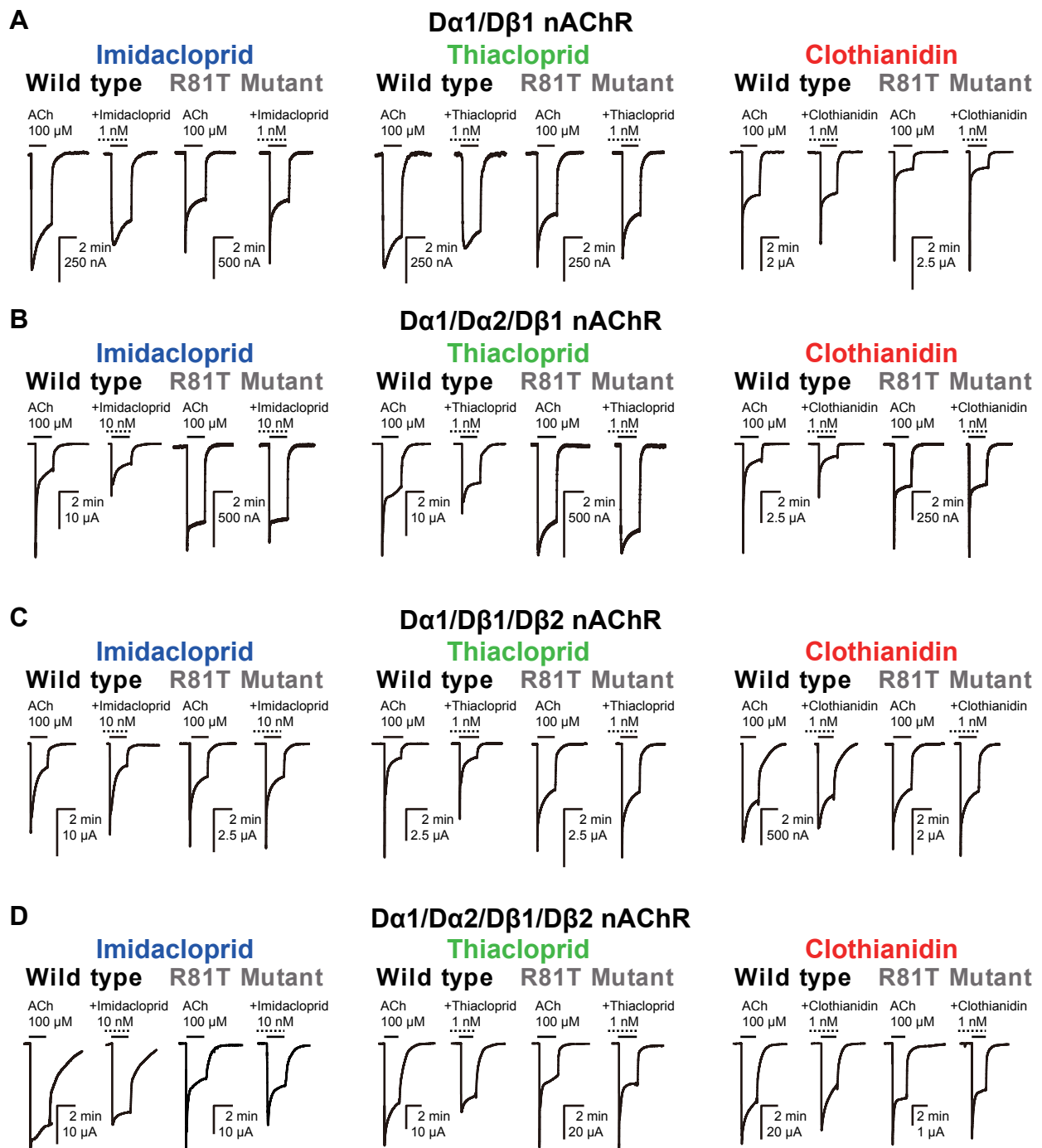
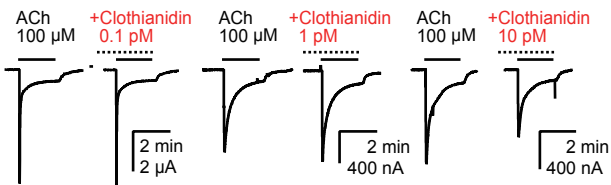
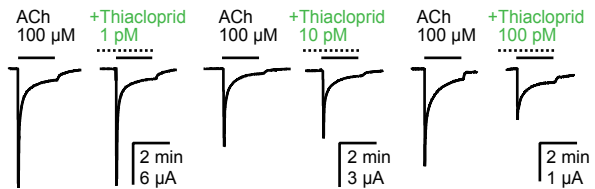
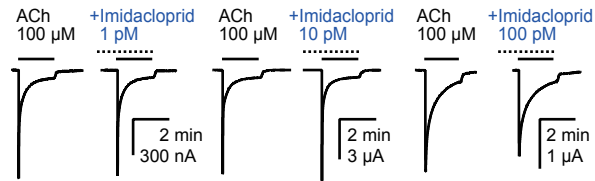
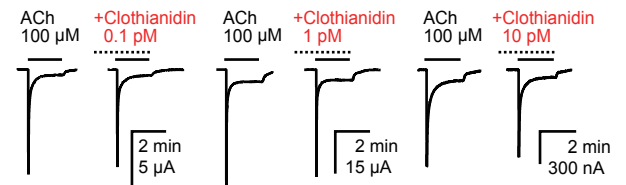
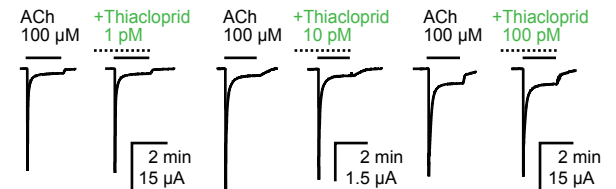
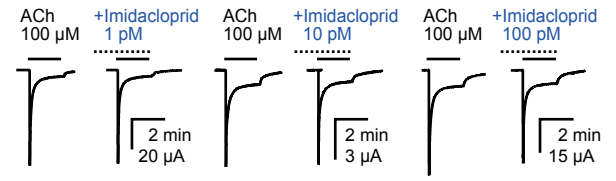


Fig. S5. Modulation by neonicotinoids of responses to 100 μM ACh of wild type and R81T mutant *D. melanogaster* nAChRs. Neonicotinoids were bath-applied for 1 min (broken horizontal bar) prior to co-application with 100 μM ACh for 2 min (solid horizontal bar). (A) Da1/Dβ1 nAChR, (B) Da1/Da2/Dβ1 nAChR, (C) Da1/Dβ1/Dβ2 nAChR, and (D) Da1/Da2/Dβ1/Dβ2 nAChR.

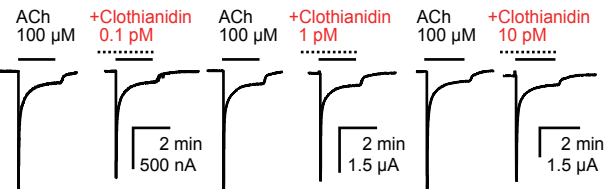
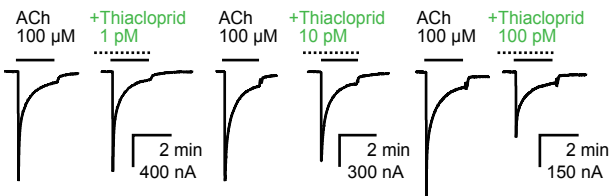
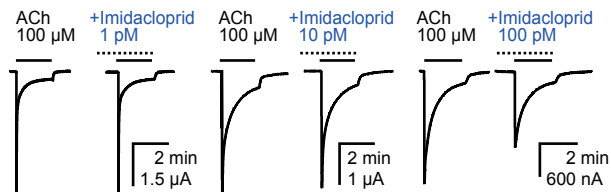
Ama1/Ama8/Amβ1 nAChR



Bta1/Bta8/Btβ1 nAChR



Ama1/Ama2/Ama8/Amβ1 nAChR



Bta1/Bta2/Bta8/Btβ1 nAChR

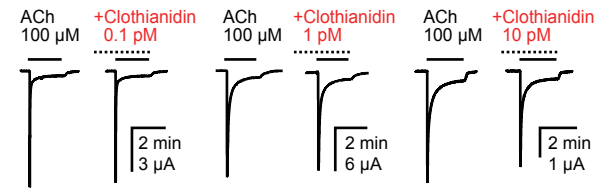
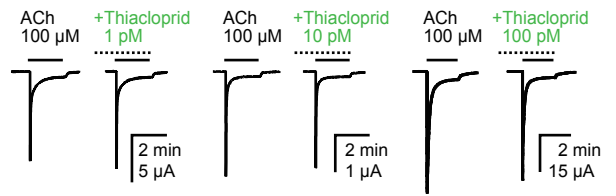
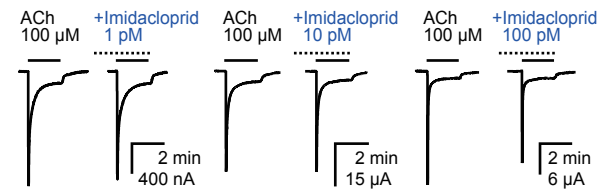


Fig. S6. Modulation by neonicotinoids of responses to 100 μM ACh of *A. mellifera* and *B. terrestris* nAChRs. Neonicotinoids were bath-applied for 1 min (broken horizontal bar) prior to co-application with 100 μM ACh for 2 min (solid horizontal bar).

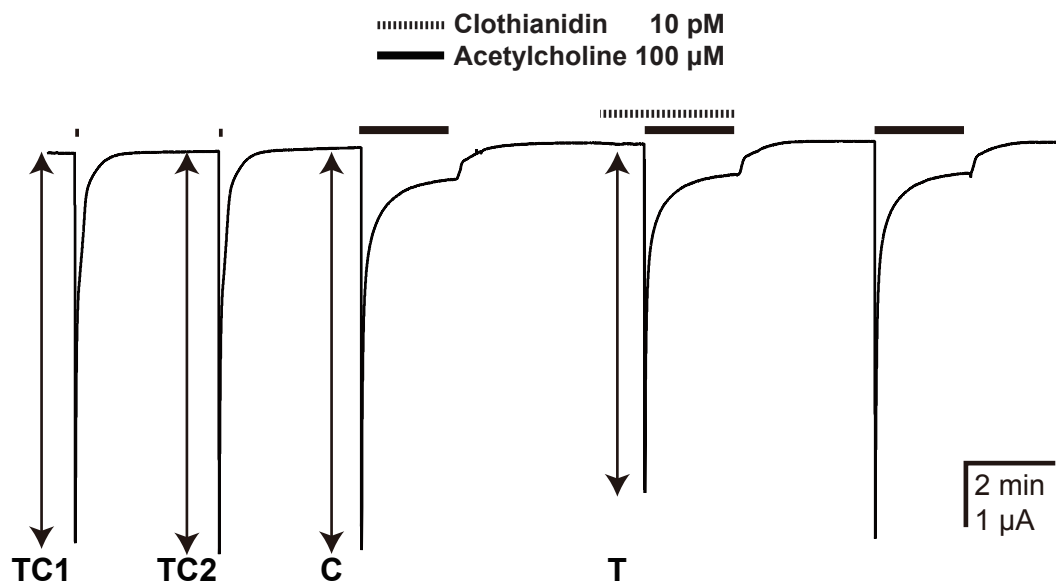


Fig. S7. Evaluation of antagonist actions of neonicotinoids on nAChRs expressed in *X. laevis* oocytes. An experiment with clothianidin on the honeybee (*A. mellifera*) nAChR (A α 1/A α 2/A α 8/Am β 1). 100 μ M ACh was successively bath-applied for 5 s at 3 min intervals. After 3 min wash, 100 μ M ACh was bath-applied for 2 min. Then, neonicotinoids were bath-applied for 1 min prior to co-application with ACh for 2 min. Horizontal bars indicate bath-application of the compounds. Normalized control [$2C/(TC1+TC2)$] and neonicotinoid-treated responses [$2T/(TC1+TC2)$] were compared.