Supporting Information

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SI Materials and Methods

Reagents. ACh, α-BgTx, DHβE, Hex, MLA, (-)-nicotine hydrogen tartrate, pyrantel citrate, (-)-tetramisole hydrochloride (levamisole), dTC, BAPTA, and BAPTA-AM were purchased from Sigma-Aldrich.

Expression Plasmids. cDNA sequences were amplified by using the following primer combinations.

acr-16 oTB361-AAACTCGAGatgtctgtctgcacccttc/oTB362-TTTGGGCCCttaggcgacaagatacggtg; *lev-1* oTB363-AAACTCGAGatgatgttaggaggtggtg/oTB364-TTTGGGCCCtcagaaaataccaagaact; *lev-8* oTB386-AAACTCGAGatgtggataccacaacgg/oTB387-TTTGGGCCCtcaggtgttaagaacgttg; *ric-3* oTB365-AAACTCGAGatgccaaaaactgaacggcg/oTB366-TTTGGGCCCtcaagtctttttaggtctc; *unc-29* oTB367-AAACTCGAGatgaggaccaaccgactatc/oTB368-TTTGGGCCCtcagggaatattggatgctg; *unc-38* oTB369-AAACTCGAGatgcgctctttttggttat/oTB370-TTTGGGCCCtcagaaactaattggattag; *unc-50* oTB371-AAACTCGAGatgagttcacagccgcgag/oTB372-TTTGGGCCCttaaagaccgccgtgttgg; *unc-63* oTB373-AAACTCGAGatgggaccaaatgaccacg/oTB374-TTTGGGCCCctaagcaagagccggcgtg; *unc-74* oTB375-AAACTCGAGatgcaaaaatatttctta/oTB376-TTTGGGCCCtcactcagctttttcgtg.

PCR fragments were then digested with XhoI and Bsp120I restriction enzymes and cloned into pTB207, an expression vector suitable for in vitro transcription and containing the 3′-UTR of the *Xenopus laevis* β-globin gene. The resulting vectors are: pTB210 *unc-29*, pTB211 *unc-38*, pTB212 *unc-63*, pTB213 *lev-1*, pTB214 *acr-16*, pTB215 *ric-3*, pTB216 *unc-74*, pTB217 *unc-50*, and pTB226 *lev-8*.

Oocyte Electrophysiology. ***BAPTA.*** In some experiments (voltage ramps and Fig. 2*A*), we used BAPTA-injected or BAPTA-loaded oocytes to avoid contamination by endogenous Ca^{2+}-dependent chloride currents. After BAPTA injection [injection of 50 nL of 40 mM K-BAPTA (pH 7.0)], oocytes were incubated in Barth solution at least 30 min (at 19 °C) before recording. For BAPTA-AM preloading, each oocyte was incubated for at least 4 h in 200 μL of Barth solution containing 100 μM BAPTA-AM (stock solution prepared at 100 mM in DMSO).

Voltage ramps. All current–voltage (*I–V*) curves were obtained with slow-voltage ramps (2-s duration) from −70 to +50 mV performed on BAPTA-injected or -loaded oocytes. For all *I–V* curves shown, leak currents recorded in the absence of agonist were subtracted from the currents recorded in the presence of agonist.

Measurement of calcium permeability. Measurements of reversal potentials were performed on oocytes preloaded with BAPTA-AM. Switching from the 1 mM $CaCl_2$ to the 10 mM $CaCl_2$ Ringer solution resulted in a voltage offset in the reference electrode because of the increase in chloride concentrations. This offset was measured for each oocyte (2.3 ± 0.2 mV, $n = 4$) and subtracted from the measured voltage in *I–V* ramps performed in 10 mM $CaCl_2$.

For calculations of P_{Ca}/P_{Na}, P_{Na} and P_K were assumed to be equal, and the internal Ca^{2+} concentration was considered negligible because of BAPTA chelation. Ionic activities rather than concentrations were used (activity coefficient of 0.72 for Na^+ and K^+ and of 0.56 for Ca^{2+} ions).

Dose–responses curves. For dose–response curves, the experimental data points of each cell were fitted with the following Hill equation: $I_{rel} = I_{max}/(1 + (EC_{50}/[A])^{nH})$, where I_{rel} is the mean relative current, I_{max} is the relative current obtained at saturating agonist concentrations, $[A]$ is the concentration of agonist, n_H is the Hill coefficient, and EC_{50} is the concentration of agonist producing 50% of the maximal current. EC_{50}, I_{max}, and n_H were fitted as free parameters. For ACh, data points were then divided by I_{max} to have a maximum relative current normalized to 1. Resulting values obtained for each individual cell and for each concentration of agonist were then averaged, and the mean data points were then fitted with the same Hill equation as above but with I_{max} fixed to 1. For levamisole, data points were normalized in the same way to the mean value of I_{max} instead of 1.

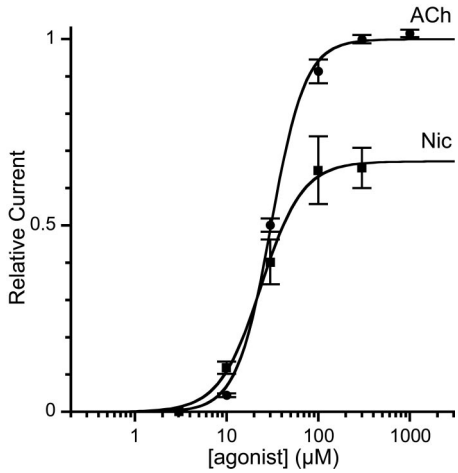

Fig. S1. ACh and nicotine dose–response curves on N-AChRs. Dose–response curves for ACh and nicotine on oocytes coinjected with the two *acr-16* and *ric-3* cRNAs are shown. EC_{50} and n_H for ACh are 31 $\pm$ 0.5 μM and 2.4 $\pm$ 0.3 ($n = 6$). EC_{50} and n_H for nicotine are 24 μM and 1.9. For nicotine, each point is the mean of 5 experimental data points, normalized to a prior application of 1 mM ACh. The maximal currents evoked by nicotine were on average 67 $\pm$ 6% of those elicited by saturating ACh concentrations.