## Appendix: Supplmentary Material

## Figure A.1. Co-release of enkephalin, galanin, NPY, and VIP neuropeptides from chromaffin cells stimulated by nicotine.

Secretion of neuropeptides from chromaffin cells (bovine) in primary culture was stimulated by incubation of cells with nicotine (10  $\mu$ M) for 15 minutes, and the media was collected for neuropeptide measurements. Secretion of Met-enkephalin, galanin, NPY, and VIP neuropeptides (panels A, B, C, and D) was stimulated by nicotine in a concentration-dependent manner. Graphs show plots of average secreted neuropeptide values among replicate cellular samples (x  $\pm$  s.e.m., replicates of n=3). Nicotine concentrations that induced half-maximal release of each of these neuropeptides, known as the EC<sub>50</sub> values, were deduced from these data and displayed in Table A.1. The EC<sub>50</sub> values for nicotine stimulation were 4.3, 2.4, 3.5, and 5.2  $\mu$ M nicotine for enkephalin, galanin, NPY, and VIP, respectively.

Figure A. 1

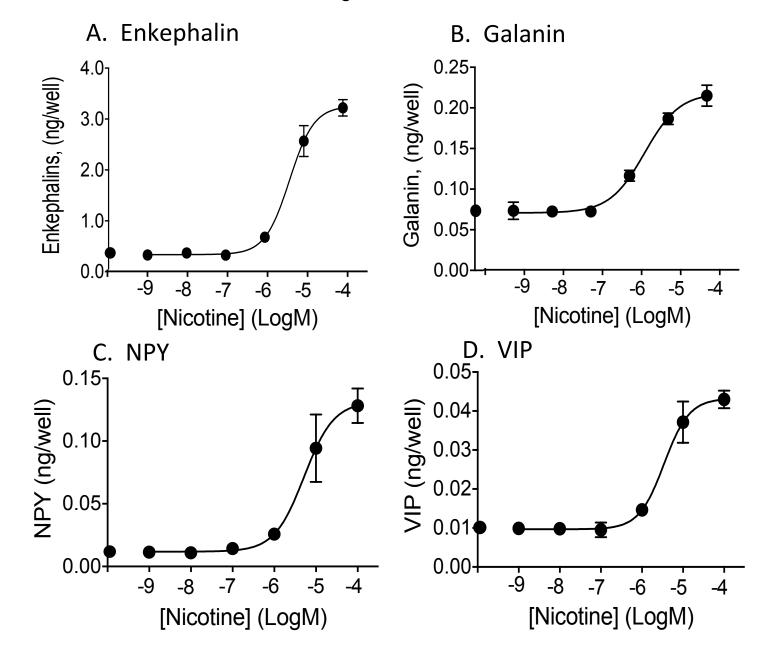


Table A.1. Nicotine stimulation of neuropeptides secreted from chromaffin cells

Neuropeptide	EC <sub>50</sub> for Nicotine-stimulated release
Met-enkephalin	4.29 <u>+</u> 0.02 μM
Galanin	2.45 <u>+</u> 0.08 μM
VIP	3.46 <u>+</u> 0.02 μM
NPY	5.19 <u>+</u> 0.02 μM

Chromaffin cells in primary culture were treated with nicotine (at different concentrations) for 15 minutes, and the media was then collected for measurements of neuropeptide levels, as described in the methods.  $EC_{50}$  values are the concentrations of nicotine that produced one-half of the maximal release of each neuropeptide.