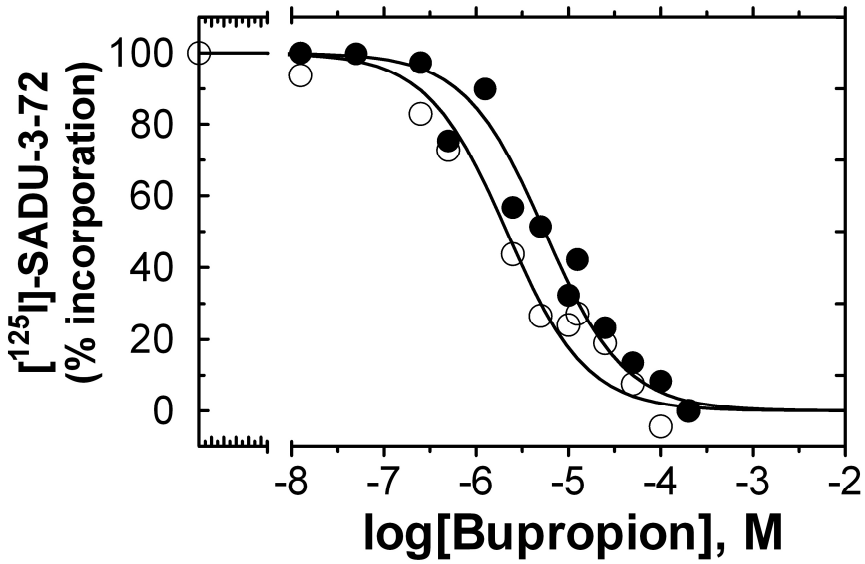
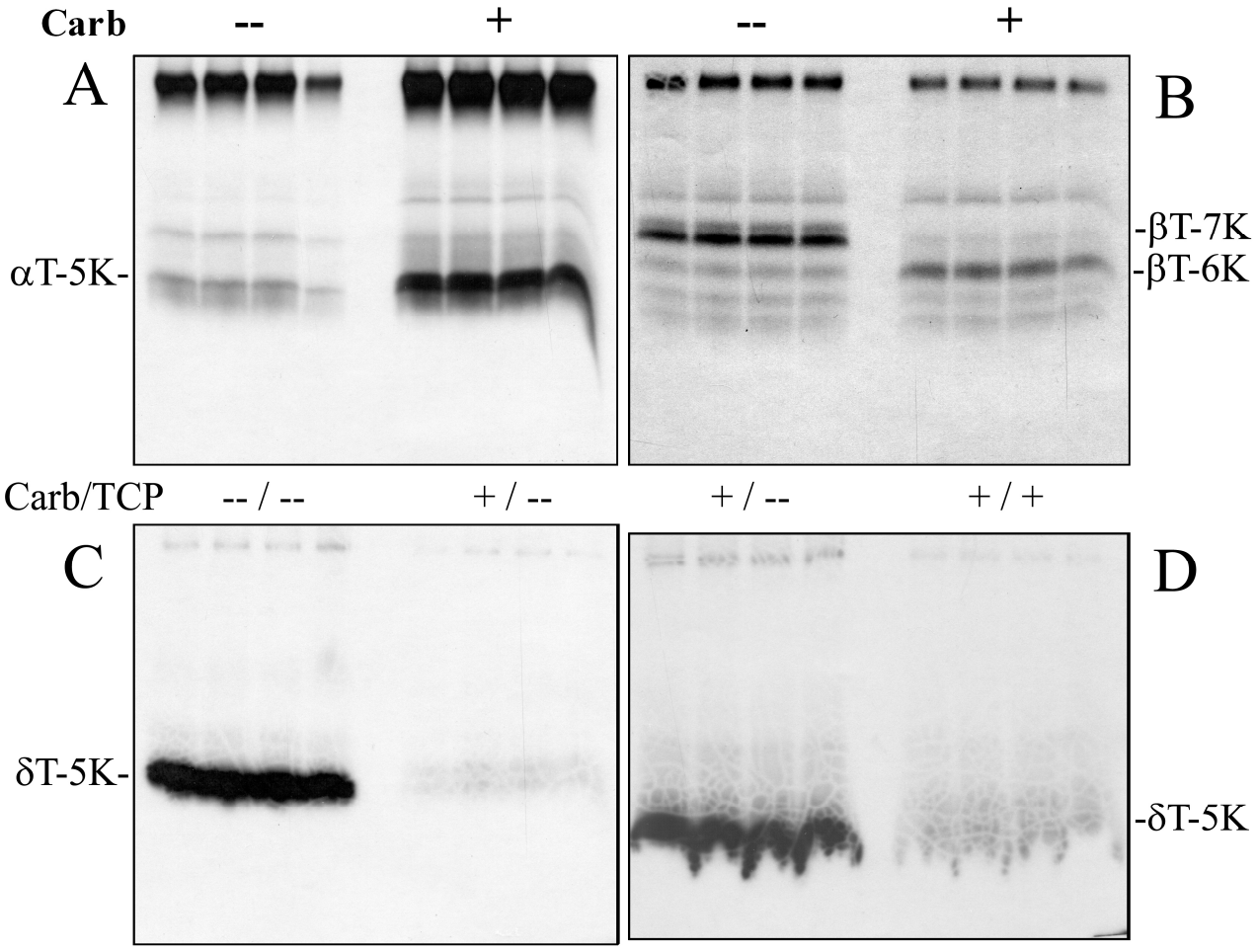
Supplementary Figure S1



S1: **Bupropion inhibition of [^{125}I]-SADU-3-72 photoincorporation into the δ-subunit of the *Torpedo* nAChR in the absence or presence of the agonist carbamylcholine.** *Torpedo* nAChR-rich membranes (~55 μg) were photolabeled with 0.2 nM [^{125}I]-SADU-3-72 (*irradiated at 365 nm for 10 min*) in the absence (●) or presence (○) of 400 μM Carb and in the presence of increasing concentrations (0 μM – 200 μM) of bupropion. nAChR subunits were resolved in an 8% SDS-polyacrylamide gel, stained, dried and processed to develop an autoradiograph (not shown). ^{125}I incorporation into the δ-subunit was determined by gamma-counting of excised gel bands after autoradiography. Normalized values for bupropion inhibition of δ-subunit labeling (● , IC_{50} = 5.5 ± 0.7 μM; ○, IC_{50} = 2.1 ± 0.7 μM) were fit to a one-site model. Total δ subunit ^{125}I incorporation was 640 (●) and 230 cpm (○) and non-specific labeling in presence of 200 μM bupropion was 80 (●) and 70 cpm (○).

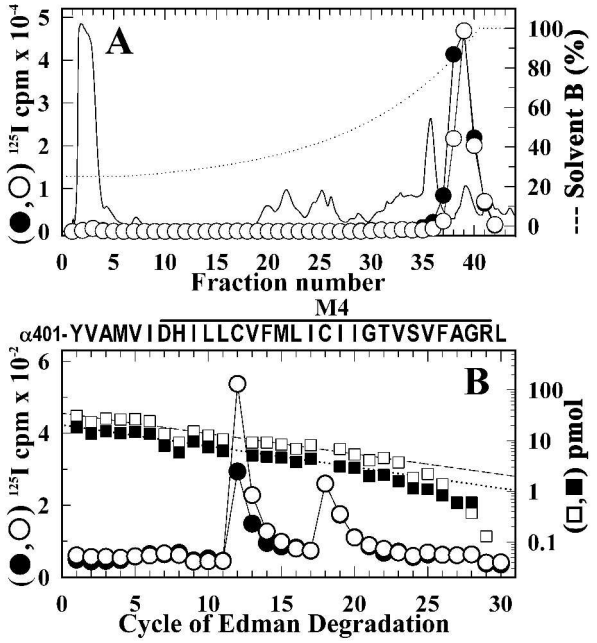
Supplementary Figure S2



S2. Tricine SDS-PAGE separation of tryptic digests of [^{125}I]-SADU-3-72-photolabeled fragments αV8-20K, βV8-22K, and δV8-14K isolated from purified *Torpedo* nAChR (A-C, + α-BgTx or + Carb) or nAChR-rich membranes in the desensitized state (+Carb, – or + TCP). *Torpedo* nAChRs were photolabeled with [^{125}I]-SADU-3-72 (7 nM (A-C) or 15 nM (D)). The α, β and δ subunits were isolated from an 8% SDS-PAGE gel and digested 'in-gel' with V8 protease, as detailed in Experimental Procedures. The labeled fragments αV8-20K, βV8-22K, and δV8-14K were isolated and further digested with trypsin. Material from each tryptic

digest was then resolved on 1.0 mm thick small pore (16.5%T/6%C) Tricine SDS-PAGE gels (*1, 2*). After Coomassie Blue R-250 staining (1 h) and destaining (3-4 h), Tricine gels were dried and exposed to film (8-12 h). The ^{125}I-containing bands (αT5K, βT7K, and δT5K) were excised from the Tricine gels and labeled material was isolated for reversed-phase HPLC purification (αT5K, Fig. 8A; βT7K, Fig. 6B; δT5K, Fig. 6A and 7A) and subsequent sequence analysis.

Supplementary Figure S3



S3. Identification of amino acids photolabeled by [^{125}I]-SADU-3-72 in the *Torpedo* nAChR αM4 segment. From the photolabeling experiment of Figures 6, α subunits were isolated from purified *Torpedo* nAChR photoabeled with [^{125}I]-SADU-3-72 in the presence of α-BgTx (●, ■) or presence of Carb (○,□) and digested 'in-gel' with V8 protease. The labeled fragment αV8-10K was isolated and further digested with trypsin. **A)** Reversed-phase HPLC fractionation of the [^{125}I]-SADU-3-72-labeled tryptic digest of αV8-10K. The elution of peptides was monitored by absorbance at 210 nm (solid line), and ^{125}I elution was quantified by γ counting of each fraction (●,○). **B**, ^{125}I (●,○) and PTH-amino acids (■, □) released during amino acid sequence analysis of ^{125}I peak (fractions 38-40; ●, 60,000 cpm; ○, 40,000 cpm) from **A**. In each sample, the primary peptide detected began at αTyr^{401} (●, I_0 = 20 ± 1 pmol, R = 91%; ○, I_0 = 35 ± 2 pmol, R = 91%) and for both samples there were peaks of ^{125}I cpm

release in cycles 12 and 18 corresponding to labeling of αCys^{412} (●, 8 cpm/pmol; ○, 9 cpm/pmol) and αCys^{418} (●, 10 cpm/pmol; ○, 6 cpm/pmol).

References:

1. Blanton, M. P., and Cohen, J. B. (1994) Identifying the lipid-protein interface of the Torpedo nicotinic acetylcholine receptor: secondary structure implications, *Biochemistry 33*, 2859-2872.
2. Schagger, H., and von Jagow, G. (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa, *Anal Biochem 166*, 368-379.