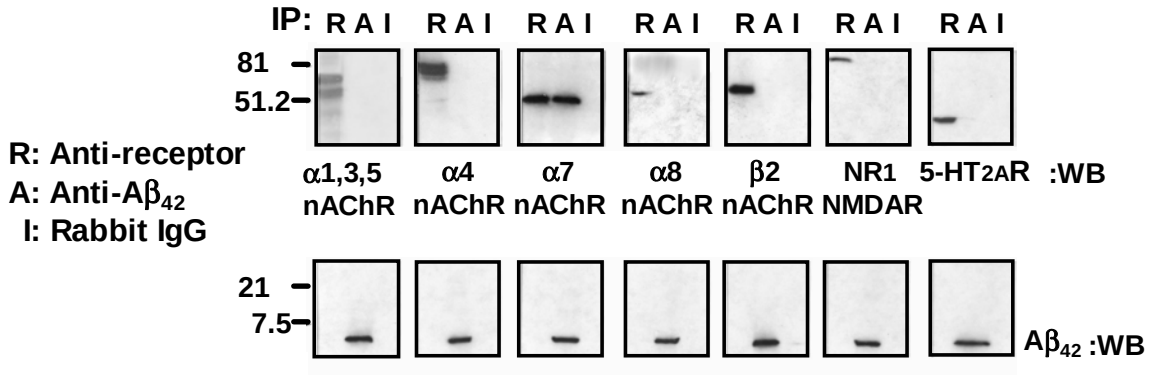
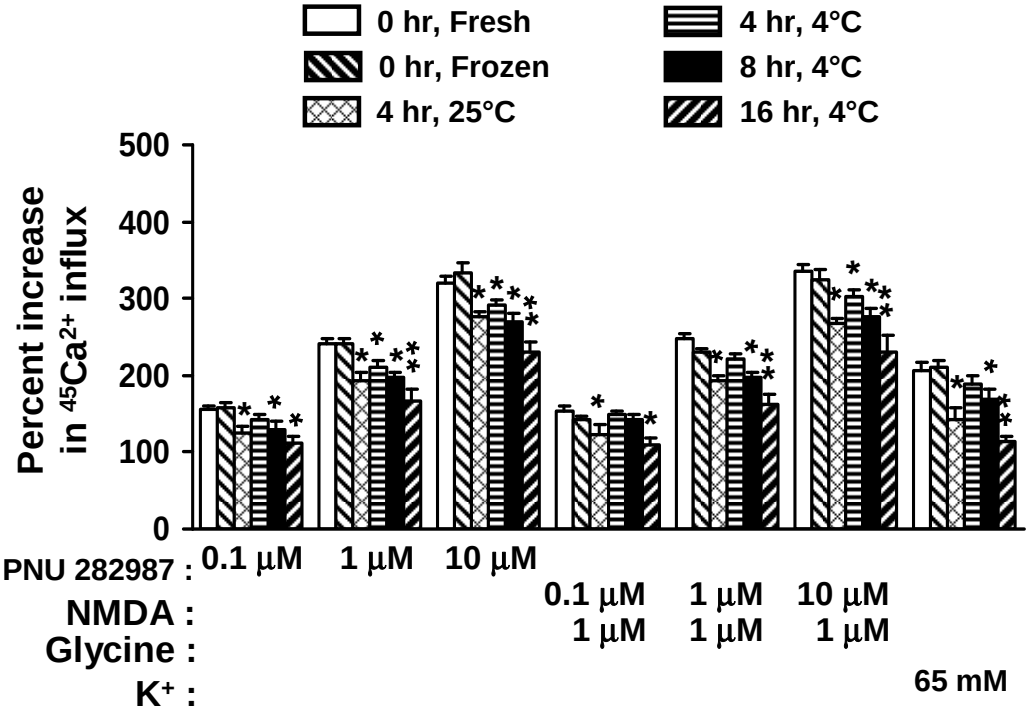
Supplementary Fig. 1



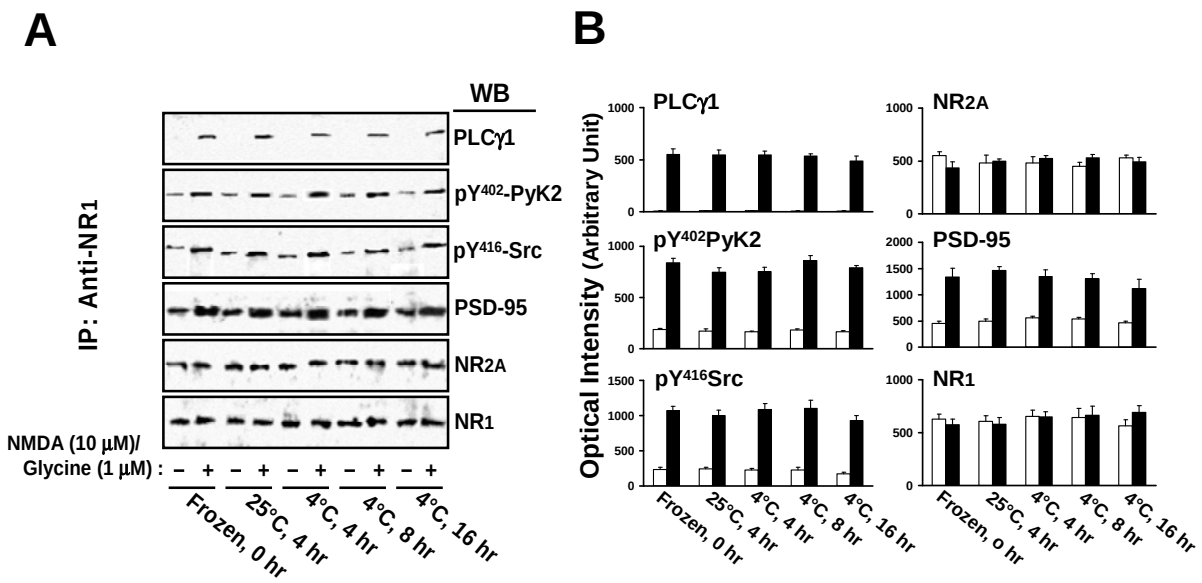
$A\beta_{42}$ interacts selectively with the α7nAChRs. Using synaptosomes prepared from rat frontal cortices, the selectivity of $A\beta_{42}$-α7nAChR interaction was determined by comparing anti-receptor immunoprecipitates (**R**) of the Kreb's-Ringer incubated synaptosomal lysate to anti-$A\beta_{42}$ immunoprecipitates (**A**) of the $A\beta_{42}$-incubated synaptosomal lysate with pre-immune rabbit IgG (**I**) as a control. Western blots using specific antibodies directed against the indicated receptors reveals that $A\beta_{42}$ selectively associated with the α7nAChRs. The selective $A\beta_{42}$-α7nAChR association shown here is consistent with the result from our previous studies (Wang et al., 2000a, b). The blots shown are the representative of 3 individual determinations from frontal cortex of one rat.

Supplementary Fig. 2



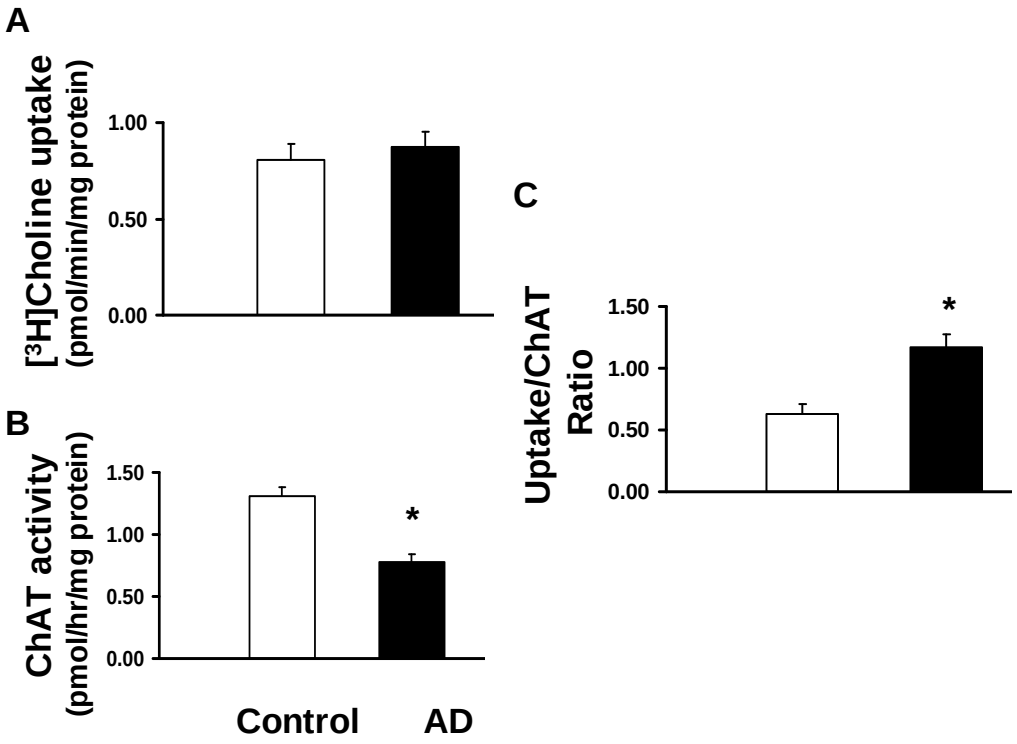
Rat frontal cortex with postmortem delays retains α7nAChR, NMDAR and voltage-gated Ca^{2+} channel responsiveness measured by stimulation-induced Ca^{2+} influx. To simulate postmortem delays, rats were sacrificed and placed at 25°C for 4-hr or 4°C for 0, 4, 8 or 16 hr. At the indicated time, brains were removed and frontal cortices were dissected, frozen immediately on dry ice and stored at -80°C for 1 week. For comparison, fresh rat brain frontal cortices (fresh 0 hr) were obtained. Synaptosomes (50 μg) prepared from these tissues were used to assess α7nAChR, NMDAR and voltage-gated Ca^{2+} channel function respectively by Ca^{2+} influx in response to 5-min 0.1-10 μM PNU 282987, 5-min 0.1 - 10 μM NMDA/ 1 μM glycine and 1-min 65 min K^+-depolarization. Basal, non-stimulated Ca^{2+} influx levels in synaptosomes from fresh 0 hr, frozen 0 hr, 25°C 4 hr, 4°C 4 hr, 4°C 8 hr, 4°C 16 hr were 5660.4 ± 321.9, 5060.2 ± 218.3, 2285.3 ± 140.1, 3600.3 ± 150.6, 3194.6 ± 157.2 and 2648.8 ± 145.3 cpm, respectively. In addition, stimulation-induced Ca^{2+} influx through α7nAChRs, NMDARs and voltage-gated Ca^{2+} channels measured by PNU 282987 and NMDA/glycine dose-dependency and K^+-depolarization responsiveness in synaptosomes are relatively stable measures in relation to the postmortem intervals. Data are expressed as means ± s.e.m of the % stimulation above basal $^{45}Ca^{2+}$ levels in cpm of 5 independent determinations each used an individual rat brain. *$p<0.05$, **$p<0.01$ compared to fresh 0 hr induced by a specific dose of stimulus (One-factor ANOVA followed by Newman-Keuls multiple comparisons).

Supplementary Fig. 3



Rat frontal cortex with postmortem delays maintains stable NMDAR signaling, NMDAR – PSD-95 coupling and assembly integrity. To simulate postmortem delays, rats were sacrificed and placed at 25°C for 4 hr or 4°C for 0, 4, 8 or 16 hr. At the indicated time, brains were removed and frontal cortices dissected, frozen immediately on dry ice and stored at -80°C for 1 week. After slowly thawing, frontal cortical slices were used to assess the effect of postmortem delay on NMDAR signaling, NMDAR – PSD-95 interaction and NMDAR assemblies following incubation with either Kreb's-Ringer or 10 μM NMDA/1 μM glycine for 30 min. The levels of PLCγ1, pY^{402}PyK2, pY^{416}Src, PSD-95 together with NR2A and NR1 in the anti-NR1 immunoprecipitate from frontal cortical slice lysates were determined by Western blotting (**A**) and quantified using densitometric scanning (**B**). Although PLCγ1 recruitment under non-stimulated basal condition was essentially undetectable, NMDA/glycine-stimulated PLCγ1 were similar among 5 time points. The NMDA/glycine-induced PyK2 and Src activation, measured by the ratio of NMDA/glycine-induced pY^{402}PyK2, pY^{416}Src and PSD-95 association did not differ, with respect to its basal level, at any time point ($p = 0.25$). Similarly, NR1 and NR2A levels were comparable among all 5 time points with or without NMDA/glycine stimulation. Data are expressed as means ± s.e.m. of the optical intensity in arbitrary units derived from 4 independent determinations from an individual rat brain. There was no statistical difference at any time points for any parameter examined (ANOVA).

Supplementary Fig. 4



Reduced acetylcholine synthesis with normal high-affinity choline uptake in synaptosomes of postmortem frontal cortices from AD subjects. Synaptosomes prepared from frontal cortices of 11 control/AD pairs were used to assess active high-affinity choline uptake (**A**) and ChAT activity (**B**) by incubating with 0.1 μM [^{3}H]choline containing Kreb's-Ringer for 5 and 30 min, respectively. The level of high-affinity choline uptake per active cholinergic nerve terminal in frontal cortical synaptosomes was also estimated using the high-affinity choline uptake/ChAT activity ratio (**C**). A 40.6% reduction of ChAT activity with a 2-fold increase choline uptake per viable nerve terminal was detected in AD tissues. Data are expressed as means ± s.e.m. of 11 pairs of frontal cortical synaptosomes from control and AD subjects. *$p < 0.01$ comparing control to the best matched AD by two-tailed Student's *t* test.