

Supplemental data

α -Bgtx TITLE: The novel $\alpha 7\beta 2$ -nicotinic acetylcholine receptor subtype is expressed in mouse and human basal forebrain: Biochemical and pharmacological characterisation

Milena Moretti, Michele Zoli, Andrew A George, Ronald J Lukas, Francesco Pistillo, Uve Maskos, Paul Whiteaker, and Cecilia Gotti

Supplementary figure 1: Specificity of the subunit-specific polyclonal antibodies tested in 2% TritonX-100 extracts of mouse brain tissue: Specificity was tested by immunoprecipitation in extracts of $\alpha 7^{+/+}$ and $\alpha 7^{-/-}$ mouse hippocampus of $\alpha 7$ mice, and $\beta 2^{+/+}$ and $\beta 2^{-/-}$ mouse coetex, as described in Materials and Methods. The extracts were labelled with 5 nM [125 I]- α -Bungarotoxin (α -Bgtx), or with 1 nM [3 H]-Epibatidine (Epi).

% of [125 I]- α -Bgtx labeled receptors immunoprecipitated

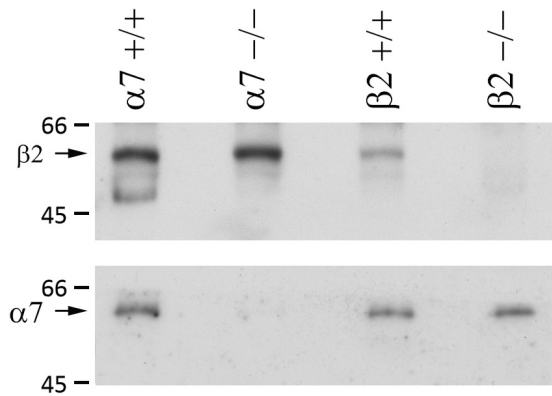
Antibodies	$\alpha 7^{+/+}$ hippocampus	$\alpha 7^{-/-}$ hippocampus	$\beta 2^{+/+}$ cortex	$\beta 2^{-/-}$ cortex
$\alpha 7$ mouse cyt	95 \pm 5 %	3 \pm 2	91 \pm 3	93 \pm 2
$\alpha 7$ COOH rat	90 \pm 2	2 \pm 2	92 \pm 2	90 \pm 2

% of [3 H]-Epi labeled receptors immunoprecipitated

Antibodies	$\alpha 7^{+/+}$ hippocampus	$\alpha 7^{-/-}$ hippocampus	$\beta 2^{+/+}$ cortex	$\beta 2^{-/-}$ cortex
$\beta 2$ human cyt (1)	95 \pm 5 %	92 \pm 2	91 \pm 3	3 \pm 2
$\beta 2$ COOH Rat	90 \pm 2	85 \pm 3	92 \pm 2	2 \pm 2

The reported values are expressed as % of specific immunoprecipitation calculated from binding to the extract, and are the mean \pm SEM of three determinations.

The same extracts were also analyzed by western blotting with the indicated Abs: $\beta 2$ (top) and $\alpha 7$ (bottom) On the left the standard molecular weight is expressed in kDa.



Supplementary figure 2: Specificity of the subunit-specific polyclonal antibodies tested in 2% TritonX-100 extracts of nAChR-expressing cell lines: Specificity was tested by immunoprecipitation of nAChR from extracts of HEK cells transfected with the $\alpha 2\beta 4$, $\alpha 4\beta 2$, $\alpha 3\beta 4$, or SH-SY5Y cells transfected with $\alpha 7$ subunits as described in Materials and Methods. The extracts were labelled with 2nM [^3H]-Epi. The reported values are expressed as % of specific immunoprecipitation, and are the mean \pm SEM of three determinations

% of [^3H]- Epi labeled receptors immunoprecipitated				
Antibodies	$\alpha 2\beta 4$	$\alpha 4\beta 2$	$\alpha 3\beta 4$	$\alpha 7$
Anti- $\alpha 2$ human	90 \pm 2 %	2	3	3
Anti- $\alpha 3$ human	2 \pm 2	0	92 \pm 2	2 \pm 2
Anti- $\alpha 4$ human	2	95 \pm 2	0	0
Anti- $\beta 2$ human	2 \pm 1	93 \pm 2	0	0
Anti- $\beta 4$ human	85 \pm 4	2	92 \pm 3	0

The same extracts were also analyzed by Western blotting and probed with the indicated Abs anti- $\alpha 7$ (top), anti- $\beta 2$ (middle) anti- $\beta 4$ (bottom). On the left the standard molecular weight is expressed in kDa

