38 and UNC-29 co-localise on the muscle cell membrane. We have co-expressed *Ascaris* UNC-38 and UNC-29 in *Xenopus* oocytes to form functional ACh-, nicotine and levamisole-gated ion channels, the first successful heterologous expression of a parasite nAChR. Changing the subunit stoichiometry produces receptor populations with different pharmacological properties: injecting different RNA ratios to drive expression towards a 3:2 UNC-38:UNC-29 stoichiometry produces a receptor population more sensitive to nicotine and oxantel, whereas driving expression to favour a 2:3 UNC-38:UNC-29 stoichiometry produces receptors more sensitive to levamisole and pyrantel. The pharmacology of these receptors resembles the L- and N-subtypes previously observed in native *A. suum* muscle cell membranes. In addition, we describe a novel nAChR subunit gene, *acr-26*, that is conserved in several evolutionary distinct parasitic species but, to date, not in any free-living or plant parasitic species. Immunofluorescence using an antibody against *A. suum* ACR-26 designed and tested in-house demonstrated expression in the head region of the nematode. Sequence data similarities with other nAChR subunits, such as the nematode ACR-16 and the vertebrate α7 and α9 subunits, combined with a computer modelling approach predicted that ACR-26 could form a homomeric receptor. We injected cRNA encoding ACR-26 into *Xenopus* oocytes and observed that a novel homomeric receptor was expressed in these cells, forming cation channels sensitive to both acetylcholine and nicotine.

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**1.3**

## Pharmacological chaperoning of nicotinic receptors begins in the endoplasmic reticulum: Compartments and stoichiometries

Rahul Srinivasan[1,*], Rigo Pantoja[1], Sindhuja Kadambi[1], Elisha D.W. Mackey[1], Shelly Tzlil[2], Fraser J. Moss[1], Henry A. Lester[1]

[1] *Division of Biology, United States*
[2] *Division of Chemistry and Chemical Engineering, Caltech, Pasadena, CA 91101, United States*

Pentameric α4β2 neuronal nicotinic acetylcholine receptors (nAChRs) assemble in two possible stoichiometries, $(\alpha4)_2(\beta2)_3$ or $(\alpha4)_3(\beta2)_2$. The proportion of the total receptor population represented by each stoichiometry in a cell is influenced by the local environment. *Se*lective *pha*rmacological *ch*aperoning of nicotinic *a*cetylcholine receptor (nAChR) *n*umber and *s*toichiometry (SePhaChARNS) is an important aspect of nicotine addiction and can explain the inadvertent therapeutic effects of smoking in Parkinson's disease. We employed fluorescent protein (FP)-tagged nicotinic acetylcholine receptor (nAChR) subunits to study the effects of nicotine and cytisine on: (1) intracellular receptor stoichiometry using pixel-by-pixel Förster Resonance Energy Transfer (FRET) and (2) trafficking of assembled nAChRs to the plasma membrane (PM) by total internal reflection fluorescence microscopy (TIRFM). Neuroblastoma (N2a) cells were transiently co-transfected with α4mCherry and β2GFP nAChR subunits. Nicotine (1 μM for 4 h) incubation increased the assembly of the $(\alpha4mCherry)_2(\beta2GFP)_3$ nAChR stoichiometry. Subcellular stoichiometry analysis revealed that nicotine induced preferential $(\alpha4mCherry)_2(\beta2GFP)_3$ receptor assembly in the endoplasmic reticulum (ER). TIRFM showed that nicotine exposure restricted localization of the newly assembled $(\alpha4GFP)_2(\beta2)_3$ receptors to the ER. Conversely, cytisine treatment (1 μM for 4 h) of α4mCherry and β2GFP transfected N2a cells resulted in preferential assembly of the $(\alpha4mCherry)_3(\beta2GFP)_2$ stoichiometry in the ER and an increase in surface trafficking of assembled nAChRs relative to non-treated controls. To study the influence of β2 on α4β2 nAChR trafficking, N2a cells were transiently co-transfected with either α4GFPβ2 or α4GFPβ4 and imaged 48 h post-transfection by TIRFM. mCherry with a lyn kinase membrane localization signal was included in transfections to visualize the PM. The α4GFPβ2 receptors trafficked to the PM in ~10 % of the cells while ~90% of cells displayed α4GFPβ4 at the PM. Together, these data reveal a rate-limiting role for β2 subunits in ligand-induced α4β2 nAChR trafficking and stoichiometry-based differences in subcellular receptor localization.

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**1.4**

## Pharmacological chaperoning of nicotinic receptors begins in the endoplasmic reticulum: High-resolution imaging

Rigo Pantoja[1,*], Rahul Srinivasan[1], Sindhuja Kadambi[1], Elisha D.W. Mackey[1], Shelly Tzlil[2], Fraser J. Moss[1], Henry A. Lester[1]

[1] *Division of Biology, United States*
[2] *Division of Chemistry and Chemical Engineering, Caltech, Pasadena, CA 91101, United States*

Nicotine addiction is the world's leading preventable cause of mortality. Smokers also have a much lower incidence of Parkinson's disease. Previous experiments show that ligand interactions with α4- and β2-nicotinic receptor subunits are necessary and sufficient for nicotine addiction. A plausible cellular/molecular mechanism for some responses to nicotine exposure is *se*lective *pha*rmacological *ch*aperoning of *a*cetylcholine *r*eceptor *n*umber and *s*toichiometry (SePhaChARNS). To investigate SePhaChARNS in a neuronlike environment, we used single-molecule resolution fluorescence microscopy to monitor localization and trafficking of α4GFPβ2 and α4GFPβ4 receptors expressed in mouse neuroblastoma (N2a) cells. As in previous investigations on native neurons and heterologous expression systems, we find large pools of endoplasmic reticulum (ER) localized α4GFPβ2 receptors. Strikingly, cells expressing α4GFPβ4 display plasma-membrane (PM) localized receptors. Pharmacological chaperoning was investigated by incubating N2a cells expressing α4GFPβ2 receptors in nicotine or the partial agonist cytisine. Furthermore, we simultaneously imaged α4GFPβ2 receptors and a mCherry-tagged ER exit site (ERES) marker to monitor the ER exit of α4GFPβ2 receptors and the associated changes in ERES. Nicotine induced an increase of ER localized α4GFPβ2 receptors, and ERES activity did not change markedly. In contrast, cytisine treatment increased the number of ERES fusion events, which may be an ERES adaptive response to increased cargo load. Consistent with an increase in cargo load we observed increased α4GFPβ2 receptor PM localization. Data from cytisine and nicotine treatments directly indicate that pharmacological chaperoning is initiated in the ER. Nicotine and cytisine induce assembly of differential α4β2 nicotinic receptor stoichiometries (see accompanying abstract by Srinivasan et al.), which leads to differential receptor localization, trafficking and ERES response. Thus, high-resolution imaging of SePhaChARNS is providing data required to understand, and manipulate nicotinic receptors with drugs.

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**1.5**

## Cloning and functional expression of the nicotinic acetylcholine receptor chaperone RIC-3 from *Xenopus laevis*

Andrew Jones

*MRC Functional Genomics Unit, Department of Physiology Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, United Kingdom*

*Xenopus laevis* oocytes provide a heterologous expression system convenient for the functional analysis of recombinant ligand-gated ion channels such as nicotinic acetylcholine receptors (nAChRs) [1]. RIC-3, first identified in *Caenorhabditis elegans*, is a chaperone protein which enhances the expression of certain nAChR subtypes. A recent study examining *Drosophila* and human cell lines showed host-cell specific effects of RIC-3 [2]. To facilitate the study of nAChR function in Xenopus oocytes, we cloned the full length *Xenopus laevis ric-3* (*Xric-3*). As with the human *ric-3*, *Xric-3* possesses two membrane spanning regions and a coiled coil domain. We found that *Xric-3* increased the current amplitude of the human α7 nAChRs and its *C. elegans* homolog, ACR-16, when co-expressed with receptor RNA in Xenopus oocytes without affecting agonist potency. Our findings suggest that the host cell specificity of RIC-3 enhancement of α7-like nAChRs, although a feature of differentiated cells is less apparent in oocytes.

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**1.6**

## Functional expression of an α5β2 nicotinic acetylcholine receptor

Tanguy Araud[1,*], Mario Wanischeck[2], Roberta Benfante[3], Ortrud Steinlein[2], Diego Fornasari[3], Daniel Bertrand[1], Jean-Charles Hoda[1]

[1] *Department of Neurosciences, Medical Faculty, University of Geneva, Geneva, Switzerland*
[2] *Institute of Human Genetics, University Hospital, Ludwig Maximilians University, Munich, Germany*
[3] *Department of Pharmacology, School of Medicine, University of Milan, CNR-Institute of Neurosciences, Milan, Italy*

The α5 subunit belongs to the family of nicotinic acetylcholine receptors (nAChRs), which are ligand-gated, cation selective channels. The α5 is predominantly expressed in limbic and autonomic regions [1–3]. Although α5 subunits are apparently associated with β2 or β4 subunits in neurons [1], no functional expression has been reported to date. It has, therefore, been suggested that these subunits merely act as accessory subunits [4]. Here. we report for the first time expression of functional nAChRs with only α5 combined with β2 or β4 subunits. Functionality of the human α5 was observed only when the cDNA encoding for this subunit contained its adjacent untranslated region (UTR). When expressed in Xenopus oocytes α5 containing receptors (α5β2 or α5β4) displayed robust currents in response to ACh and were typically in the μA range. The α5β2 nAChR displayed a high sensitivity to ACh with an $EC_{50}$ of $1.63 \pm 0.15$ μM which is about 10-fold more sensitive than the major brain α4β2 receptors. These receptors also displayed a high sensitivity to nicotine with an $EC_{50}$ of $0.58 \pm 0.17$ μM. Altogether these data demonstrate that functional receptors can be obtained with a binary combination of α5 with a β subunit and that these receptors may play an important physiological role both in the central and peripheral nervous system. The high degree of correlation between smoking and cancer in human with α5 and its non-synonymous single nucleotide polymorphism further calls for a better understanding of the functional role of this subunit. These data open new avenues for the screening of the physiological properties of α5 containing nAChRs.

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**1.7**

## Gain of function mutants in human or mouse nAChR β3 subunits interchangeably activate either human or mouse α6β4*-nAChR, but not human or mouse α6β2*-nAChR

Bhagirathi Dash[1,*], Minoti Bhakta[1], Paul Whiteaker[1], Jerry A. Stizel[2], Yongchang Chang[1], Ronald J. Lukas[1]

[1] *Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ, United States*
[2] *Department of Integrative Physiology, Institute for Behavioral Genetics, University of Colorado, Boulder, CO, United States*

It has been difficult to demonstrate function of heterologously expressed nAChR containing α6 and β3 subunits. Recently Dash et al. [1] confirmed that wild type, human nAChR β3 subunits have a dominant-negative effect on the function of α6β4*-nAChR subtypes but also found that β3 subunit gain of function mutant(s) potentiate function of human α6β4*-nAChR. Function of human α6β2*-nAChR was absent in the presence of either wild type or gain of function β3 subunits. This is puzzling, because there is function of α6β3*-nAChR containing wild type β3 subunits in rodents. In order to better understand α6β3*-nAChR, we interchangeably expressed mouse or human wild type or gain of function β3 subunits with human or mouse α6 and β2 or β4 subunits and determined functional features of expressed receptors. Gain of function mutants (M2 second transmembrane domain 9' or 13' positions) in either human or mouse β3 subunits potentiate function of either human or mouse α6β4*-nAChR but not of human or mouse α6β2*-nAChR. However, there was no function in oocytes expressing human α6 and either β2 or β4 subunits along with wild type, mouse β3 subunits. Similarly, wild type, human β3 subunits failed to produce functional receptors when