Two different hypotheses have been proposed to explain nicotine-induced up-regulation of α4β2 nAChRs. One view postulates that nicotine alters the functional state of assembled receptors in the cell surface to a state that binds more [^{3}H]epibatidine, is more sensitive to activation, and desensitizes more slowly (Buisson and Bertrand, 2001; Vallejo et al., 2005). The other view proposes that nicotine, which is membrane permeant, acts within the endoplasmic reticulum to promote the assembly and maturation of nAChRs (Wang et al., 1998; Nashmi et al., 2003; Kuryatov et al., 2005; Sallette et al., 2005). Our findings generally support the view that nicotine influences assembly and maturation of HS α4β2 nAChRs for the following reasons:

1. Oocytes injected with α4/β2 subunit cDNA ratios that produce a mixture of HS and LS α4β2 nAChRs expressed a homogeneous population of HS α4β2 nAChRs when exposed to nicotine before the initiation of receptor assembly.
2. Nicotine up-regulated HS α4β2 nAChRs in oocytes expressing predominantly LS α4β2 nAChRs without altering the function of LS α4β2 nAChRs. We were surprised to find that maximal [^{3}H]cytisine binding to oocytes injected with a 10:1 α4/β2 cDNA ratio was not increased by nicotine, which should have occurred if nicotine enhanced intracellular receptor maturation (Kuryatov et al., 2005; Sallette et al., 2005; Corringer et al., 2006). One explanation for this could be that if a key step in up-regulation of HS α4β2 nAChRs were the interaction of nicotine with an immature species such as βαβ (Corringer et al., 2006), then low levels of β2 subunit would slow the process of nicotine-driven maturation of HS α4β2 nAChRs.
3. Exposure to nicotine did not affect the biphasic ACh concentration-response curve of microtransplanted α4β2 nAChRs; i.e., nicotine did not seem to alter the sensitivity to activation of α4β2 nAChRs that were already assembled. We recognize, of course, that our conclusion is tied to the assumption that microtransplantation does not alter the molecular basis of sensitivity to up-regulation by nicotine. Although we have no direct evidence, it seems unlikely that the process of microtransplantation would alter the molecular properties of α4β2 nAChRs so drastically as to render them insensitive to nicotine up-regulation. This view is supported by the finding that neither the ACh sensitivity nor the relative proportions of HS and LS α4β2

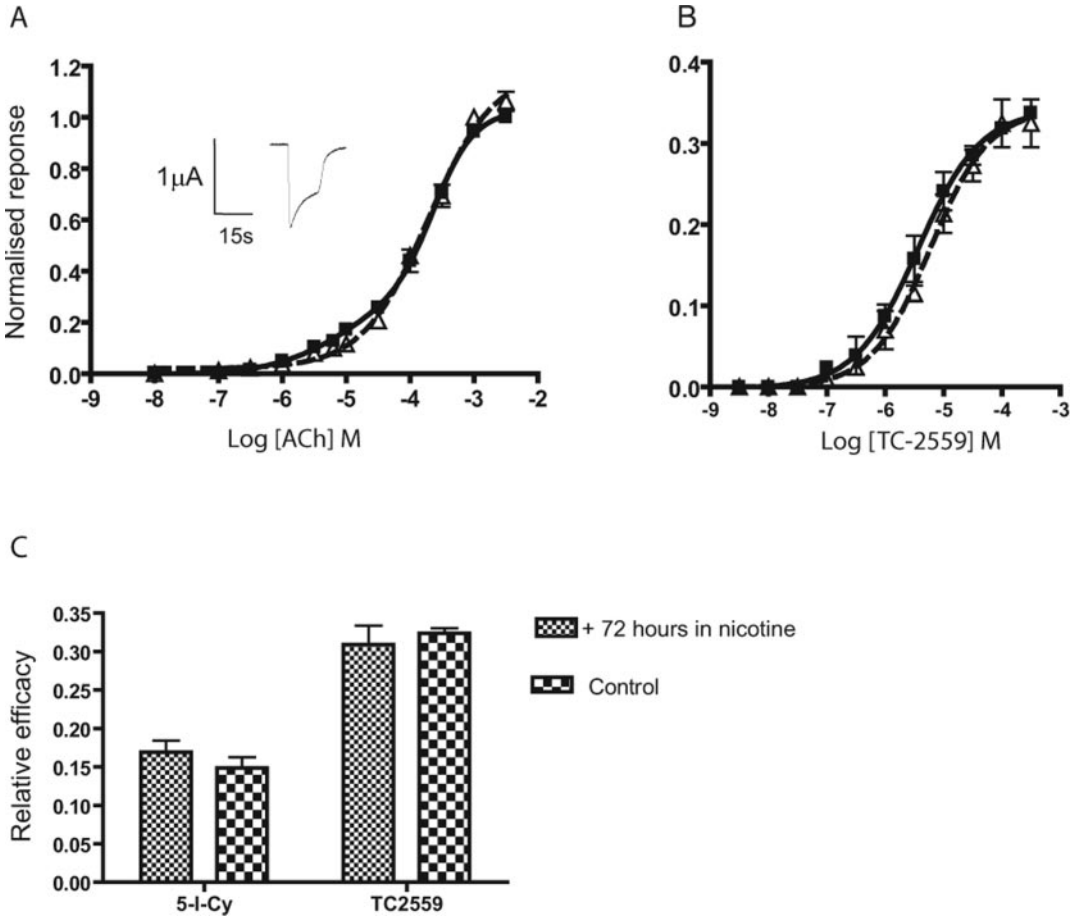

Fig. 7. Effects of long-term nicotine exposure on oocytes expressing microtransplanted human α4β2 nAChRs. The receptors were assembled and expressed first in HEK293 cells stably expressing human α4β2 nAChRs. Oocytes expressing microtransplanted α4β2 nAChRs were incubated in nicotine for 72 h and then used for electrophysiological experiments as described under *Materials and Methods*. A, ACh concentration response data for both control and nicotine-treated oocytes were best fitted to a two-component sigmoidal equation ($p < 0.001$, F test, $n = 6$). Control oocytes, – –△– –; nicotine-incubated oocytes: —■—. Inset shows a typical current response to 1 mM ACh in HEK293 cell stably expressing human α4β2 nAChRs. B, concentration-response curve for TC-2559 at control (– –△– –) and nicotine-exposed oocytes expressing microtransplanted human α4β2 nAChRs (—■—). C, bar graph showing the changes in the maximal responses to TC-2559 and 5-I-cytisine in oocytes exposed to nicotine for 72 h and control oocytes. Efficacy values shown represent data normalized to the amplitude of currents elicited by 1 mM ACh.

nAChRs expressed in the HEK293 cells were altered by microtransplantation.

To our knowledge, the question of whether both $\alpha4\beta2$ nAChR types occur in neurons has not been resolved. However, it is noteworthy that the functional effects of the $\alpha4\beta2$-preferring agonist A-85380 at HS and LS $\alpha4\beta2$ nAChRs are strikingly similar to the effects of this compound in the mouse thalamus, which may coexpress both receptor types (Butt et al., 2002). Moreover, the effects of cytisine, epibatidine and nicotine on HS and LS $\alpha4\beta2$ nAChRs receptors were comparable with the effects of these compounds on the "more DHβE-sensitive" and "less DHβE-sensitive" $\beta2$-containing receptors that are widely distributed in the mouse brain, suggesting that both stoichiometries may coexist in the brain (Marks et al., 1999). Thus, our approach of expressing homogeneous populations of HS or LS $\alpha4\beta2$ nAChRs in combination with the use of stoichiometry-selective $\alpha4\beta2$ ligands may be a first step toward the identification of the $\alpha4\beta2$ stoichiometries present in the brain and the design of stoichiometry-specific $\alpha4\beta2$ nAChRs ligands.

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