$GABA_A$ receptors increase their conductance through novel protein interactions

M.L. Tierney,[1] A.B. Everitt,[1] V.A.L. Seymour,[1] J. Curmi[1] and D.R. Laver,[2] [1]JCSMR, Australian National University, ACT 0200, Australia and [2]School of Biomedical Science, University of Newcastle, NSW 2308, Australia.

Native $GABA_A$ channels display a single-channel conductance ranging between ~10-90 pS. Diazepam increases the conductance of some of these native channels but never those of recombinant receptors unless they are co-expressed with GABARAP. This trafficking protein clusters recombinant receptors in the membrane suggesting that high-conductance channels arise from receptors that are at locally high concentrations. The amphipathic (MA) helix that is present in the large cytoplasmic loop of every subunit of all ligand-gated ion channels mediates protein-protein interactions. Here we report that when applied to inside-out patches, a peptide mimicking the MA helix of the γ2 subunit (γ381-403) of the $GABA_A$ receptor abrogates the potentiating effect of diazepam on both endogenous receptors and recombinant $GABA_A$ receptors co-expressed with GABARAP, by substantially reducing their conductance. The protein interaction disrupted by the peptide did not involve GABARAP because a shorter peptide (γ386-403) known to compete with the γ2: GABARAP interaction did not affect the conductance of recombinant αβγ receptors co-expressed with GABARAP. The requirement for receptor clustering and the fact that the γ2 MA helix is able to self-associate support a mechanism whereby adjacent $GABA_A$ receptors interact *via* their γ2 subunit MA helices, altering ion permeation through each channel. This finding has important implications for understanding both the structural design of ligand-gated ion channels and the adaptive, dynamic means a cell invokes to amplify its signalling capacity.