## Defining $GABA_A$ receptor pharmacology and physiologies through the disruption of receptor protein interactions

*M.L. Tierney,[1] V.A.L. Seymour,[1] J. Curmi,[1] W. Xu[2] and H.C. Chan,[2] [1]The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia and [2]Epithelial Cell Biology Research Center, School of Biomedical Sciences, Faculty of Medicine The Chinese University of Hong Kong, Shatin, Hong Kong.*

$GABA_A$ receptors are the dominant inhibitory neurotransmitter-gated ion channel in the central nervous system. We have identified a novel way in which these neuronal ion channels alter their electrical response. Interactions between neighbouring, clustered $GABA_A$ receptors profoundly alter single-channel properties (conductance and kinetics), leading to a significant enhancement of channel activity ('cross-talk') (Everitt *et al.*, 2009). Interactions were identified using competitor peptides that mimic defined intracellular protein binding sites. Peptides were applied directly onto inside-out membrane patches pulled from newborn rat hippocampal neurons and single-channel currents were recorded. Combining the use of competitor peptides and single-channel recordings provided a visual insight into the dynamic nature of protein interactions that affect the activity of single $GABA_A$ ion channels. Specifically, when applied to inside-out patches, a peptide mimicking the MA helix of the γ2 subunit (γ381-403) of the $GABA_A$ receptor abrogated the potentiating effect of the drug diazepam on endogenous receptors by substantially reducing their conductance.

In addition to benzodiazepines, barbiturates, general anaesthetics and neurosteroids have all been shown to facilitate neuronal receptor cross-talk, that is, the drugs potentiate GABA-activated currents increasing both channel open probability and conductance. Such drugs however, are predicted to act on different $GABA_A$ receptor subtypes. We hypothesized therefore, that modulation of ion permeation was a general mechanism through which all $GABA_A$ receptor subtypes signal. Using a competitor peptide specific to δ-containing $GABA_A$ receptors we tested our hypothesis. GABA currents were potentiated by the general anesthetic etomidate and competitor peptides were applied to neuronal patches. Addition of the δ MA peptide but not a scrambled version or the γ MA peptide abrogated the potentiating effects of etomidate. These data support our hypothesis and are aiding our understanding of the complex interplay between drugs and ion channels and also amongst the different $GABA_A$ receptors subtypes themselves.

Everitt AB, Seymour VA, Curmi J, Laver DR, Gage PW, Tierney ML. (2009) Protein interactions involving the γ2 large cytoplasmic loop of $GABA_A$ receptors modulate conductance. *FASEB Journal* **23:** 4361-4369.