Using artificial synapses to investigate $GABA_A$ receptor kinetics

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We are interested in $GABA_A$ receptors that contain the γ1 subunit, because they are highly expressed in the central amygdala but few other brain regions. However, because subunit selective drugs are limited, it is difficult to identify the physiological role of γ1-containing receptors. The aim of this study was to investigate the electrophysiological properties of GABAergic synapses with defined subunit composition, and we achieved this by co-culturing transfected HEK cells with neurons.

Time-mated rats (e18) were euthanized with CO_2 and the embryos decapitated in accordance with approval from the University of QLD Animal Ethics Committee. Dissociated neuronal cultures were grown from the embryonic cortices, and incubated for 3-5 weeks. We then transfected HEK cells with $GABA_A$ receptors and the synaptic adhesion molecule neuroligin, and plated the HEK cells on top of the mature cortical cultures. After 1-2 days, immunofluorescent labeling showed numerous GAD65-positive puncta on the HEK cells, indicating that neurons readily formed GABAergic synapses onto the HEK cells. Robust spontaneous IPSCs were observed when the co-cultured HEK cells were voltage clamped. Cells containing α2β2γ1 $GABA_A$ receptors and neuroligin 2A had spontaneous IPSCs with an average 10-90% rise time of 8.2±1.1ms ms and monoexponential decay time constant of 67.1±7.6ms. α2β2γ2L receptors were faster (rise 4.0±0.4ms and decay 38.7±3.0ms). α1β2γ1 receptors had a similarly slow rise but a faster decay (rise 4.0±0.7ms, decay 19.8±3.0ms). α1β2γ2L subunits were the fastest and most similar to IPSCs usually observed in neurons (rise 1.2±0.2ms, and decay 4.0±0.8ms). In the co-culture system, the γ1 subunit promotes slow IPSC rise and decay, as does the α2 subunit (both subunits $P<0.0005$, 2-way ANOVA). It is therefore evident that subunit composition has a strong influence on GABAergic synaptic function. Further experiments will investigate whether the observed differences are due to clustering differences or underlying receptor kinetics.