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Human Muscle Nicotinic Receptor Mechanisms

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We are investigating the effect on receptor function of mutations that cause congenital myasthenic syndromes (Vincent *et al.*, 1997). In order to understand the effects on it is necessary (e.g. Colquhoun, 1998) to fit a physically realistic mechanism to single channel recordings. In principle the optimum method of fitting the rate constants of a specified mechanism is to maximise the likelihood of the entire sequence of openings and shuttings, but to do this it is necessary to use distributions that allow for missed brief events. Colquhoun *et al.* (1996) described such fitting with exact correction for missed events, and Fig. 1 exemplifies its application to steady state recording of human nicotinic receptors (tsA201 cells transfected with wild type α, β, ε and δ subunits, resolution 20 – 25 μs at 100 mV). The results were fitted with the mechanism suggested by Milone *et al.* (1997). This supposes that the two binding sites for acetylcholine (ACh) are different (but independent), and that both singly-liganded forms can open. Fig. 1 shows the distribution of open period durations from one patch (100 nM ACh) with 2702 resolved open periods. The continuous line was *not* fitted to the data in the histogram, but is the Hawkes-Jalali-Colquhoun (1992) (HJC) distribution of apparent open times with a 25 μs resolution, and it was calculated from the rate constants for the mechanism, which were estimated as above. The three components expected in the asymptotic distribution are visible. The good fit shows that the mechanism is consistent with the observations. The dashed line shows the prediction of what the distribution of open times would be with perfect resolution. Many brief openings (mean duration about 12 μs) are missed so correction for missed events is essential. The estimated rate constants provided a similarly good fit to the distribution of apparent shut times, and the distributions of apparent open times conditional on the duration of adjacent shut times. This provides a baseline for the interpretation of the effects of mutations.

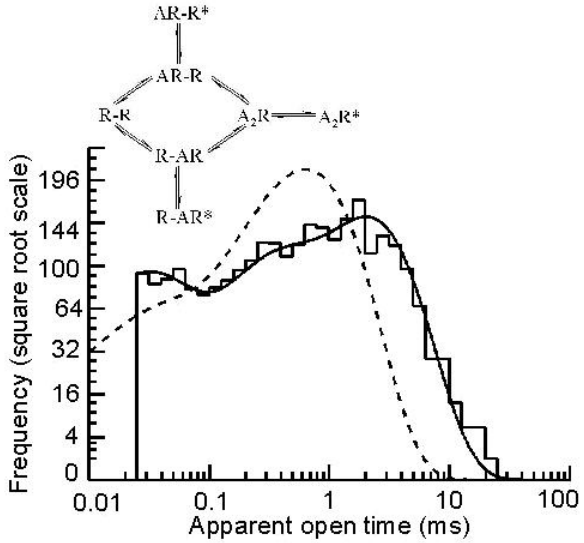

Figure 1. The distribution of apparent open period durations.

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