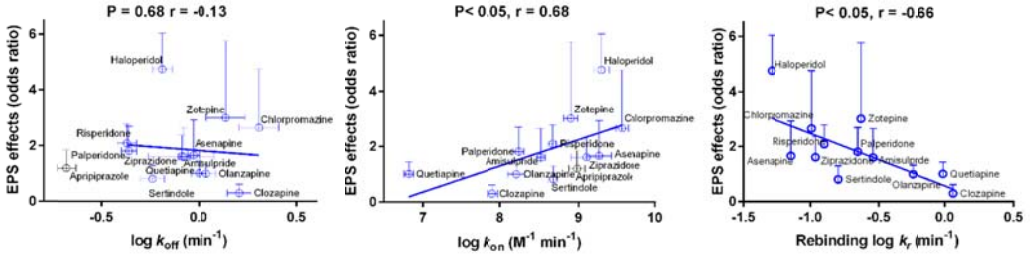
A Role For Association Kinetics In Dictating The On-target Extrapyramidal Side Effects Of Antipsychotic Dopamine D_2 Receptor Antagonists

One theory explaining why atypical dopamine D_2 antipsychotics (APs) (e.g. clozapine) show reduced extra-pyramidal side effects (EPS) involves their rapid dissociation from the dopamine D_2R, permitting an antipsychotic (AP) effect whilst accommodating physiological dopamine neurotransmission[1]. However, this hypothesis is limited to observations of the behavior of a relatively small number of dopamine APs. In order to further explore this hypothesis we have developed a novel time-resolved fluorescence resonance energy transfer (TR-FRET)-based method to measure the kinetic properties of a series of clinically relevant typical and atypical dopamine D_2 receptor antagonists. The kinetic parameters of unlabeled compounds were assessed using a competition kinetic binding assay as previously described.[2] Specifically PPHT-red (12.5 nM, final assay volume 40 μL) was incubated in the presence of unlabeled antagonist, and terbium-labelled CHO-D2L cell membranes (2 μg well^{-1}) the presence of GppNHp (100μM) at 37 ^{o}C. Specific TR-FRET binding, defined by haloperidol (10 μM) was plotted against time and fitted to a competitive kinetic binding model (GraphPad Prism 6.0).

To assess the clinical relevance of the measured kinetic rate constants we have correlated these values with clinical findings taken from a recent study detailing the relative efficacy and tolerability of a diverse group of clinical APs using a multiple-treatments meta-analysis.[3] The dissociation rate (k_{off}) of D_2 clinically relevant APs could be readily correlated with their increased incidence of prolactin secretion in the clinic, as predicted by the 'rapid dissociation hypotheses' (data not shown). Surprisingly however, k_{off} was not correlated with propensity to cause EPS in the clinic (see Fig 1), which was instead significantly correlated with the kinetic association rate (k_{on}). We therefore employed a rebinding model that takes into account the unique tissue microenvironment of the synapse and integrates both the association and dissociation rates to calculate the overall rate of reversal of receptor blockade (k_r).[4]

Fig 1. Correlation of AP EPS with kinetic association rate (k_{on}) and rebinding (k_{off})



This rebinding model better predicts the incidence of EPS and suggests that k_{on} in conjunction with k_{off} is a critical component defining the tolerability of D_2 APs. Thus the ideal D_2 AP pharmacological profile should be achievable through careful optimization of these two critical kinetic parameters.

(1) Kapur & Seeman (2001) Am J Psych. **158**, 360–369.
(2) Herenbrink et al., (2016). Nat Chem Biol. In Press.
(3) Leucht et al., (2013) Lancet. **382**, 951–962.
(4) Vauquelin & Charlton (2010) Br J Pharmacol, **161**, 488–508.