**Cholinergic muscarinic type 4 receptor is the dominant driver of Xanomeline’s efficacy**

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**Introduction.** Antipsychotics, whilst effective in supressing positive symptoms, remain completely ineffective for treating negative symptoms and cognitive deficits as seen in patients suffering from schizophrenia. Hence there is an unmet need for new therapeutics with broader spectrum of action. Xanomeline, a cholinergic muscarinic type 1 (M1) and type 4 (M4) receptors preferring agonist, has recently emerged as a new non-dopaminergic antipsychotic that successfully passed Phase III clinical trial. We hypothesised that Xanomeline produces its effect mainly via acting on M4 receptor due to recent preclinical studies showing that compounds targeting this receptor subtype (e.g., M4 positive allosteric modulators) already demonstrated therapeutic potential.

**Aims**. We validate this hypothesis using M4 receptor knockout (KO) mice in behavioural tests.

**Methods**. We conducted open-field locomotor activity (LMA) and prepulse inhibition (PPI) of acoustic startle tests in wild type and M4 KO mice (n = 7 – 15/gender/genotype), with a NMDA non-competitive blocker, MK-801, as the psychotomimetic, which induced locomotor hyperactivity, and disruption of PPI.

**Results.** In LMA test, Xanomeline (1 or 10mg/kg) significantly reversed hyperactivity in the wildtype mice. In contrast, in M4 KO mice, only the highest dose of Xanomeline (10mg/kg) could reverse the MK-801 effect. In PPI test, Xanomeline dose-dependently rescued the disruption of PPI in wildtype animals, but its effect was completely abolished in the KO mice, even at the highest dose.

**Discussion.** We demonstrated the action of Xanomeline involves different balance of M1/M4 receptors engagement. For instance, in LMA Xanomeline requires predominantly M4 with some M1 activity, but in PPI, only M4 activity is necessary. Next, we will investigate the role of M4 receptors in learning and memory tests to elucidate Xanomeline’s therapeutic potential on cognitive deficits in the treatment of schizophrenia.