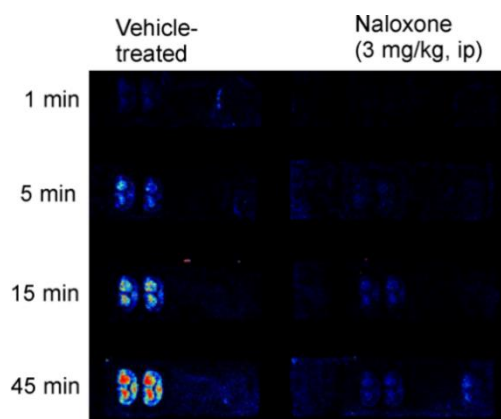


RECEPTOR OCCUPANCY – EX VIVO AUTORADIOGRAPHY

Fig. 1. Autoradiographic binding of [³H]DAMGO to striatal sections from rats given naloxone (3 mg/kg, i.p.) or vehicle, 20 minutes prior to sacrifice. After being cut, sections were incubated *in vitro* in [³H]DAMGO for between 1 and 45 minutes. Binding of [³H]DAMGO to opiate receptors in the sections from the drug-treated animal is reduced at all incubation time points, due to occupancy of opiate receptors in the tissue by the drug.

(a) Autoradiographic image



(b) ROI quantification of radiotracer binding

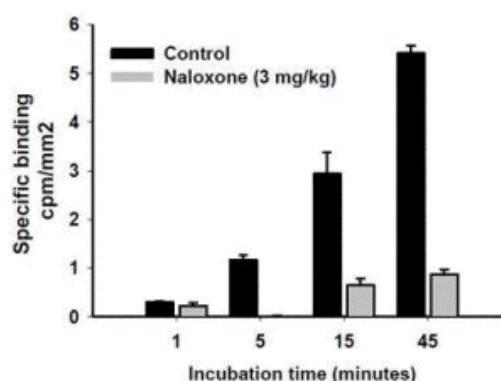
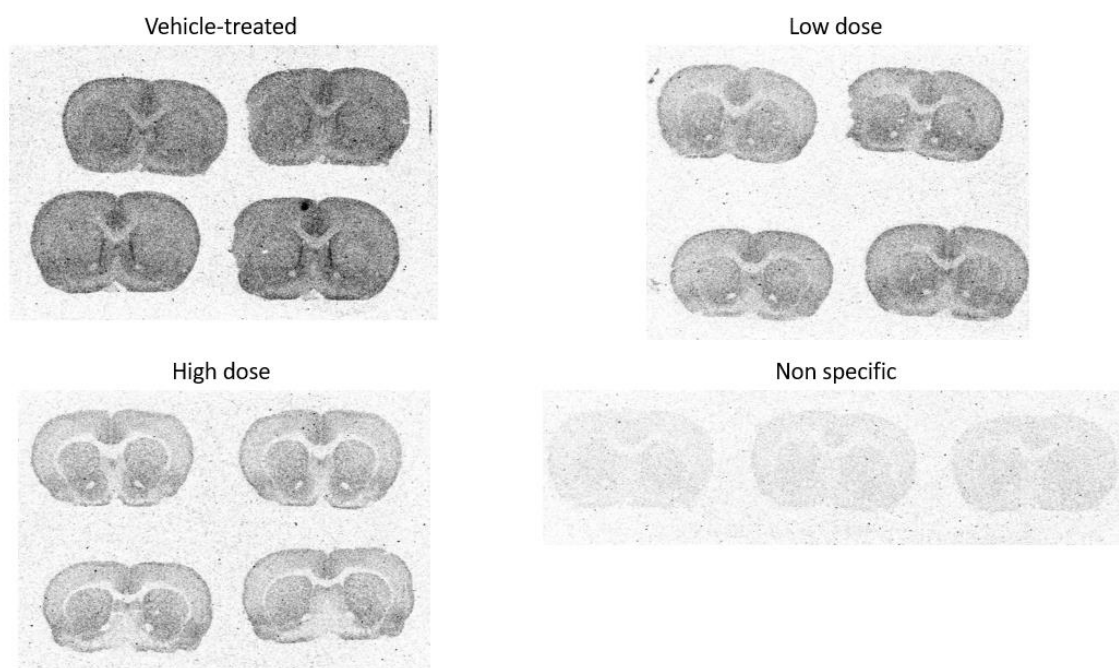


Fig. 2. Inhibition of binding of a P2X7 receptor radioligand in brain sections from rats given a P2X7 receptor-active drug at either a low dose (n=2) or high dose (n=2). Radioligand binding to the sections was significantly reduced in all 4 drug-treated animals compared to that in the vehicle controls.



RECEPTOR OCCUPANCY – TISSUE DISSECTION AND COUNTING

Fig. 1. *In vivo* CB1 receptor occupancy of a test drug determined by inhibition of [³H]SR141716A binding in the rat brain. The test drug was given by oral gavage 1 hour prior to sacrifice and [³H]SR141716A via a tail vein 30 minutes prior to sacrifice. Plotted values are ratios of radioactivity in a receptor-rich region (cerebellum or hippocampus) relative to that in a receptor-poor reference region (brain stem) and are the means of 5 - 6 animals per dose. At the highest dose, test drug reduced both hippocampal:brain stem and cerebellum:brain stem ratios to close to one. This indicates complete occupancy of brain CB1 receptors.

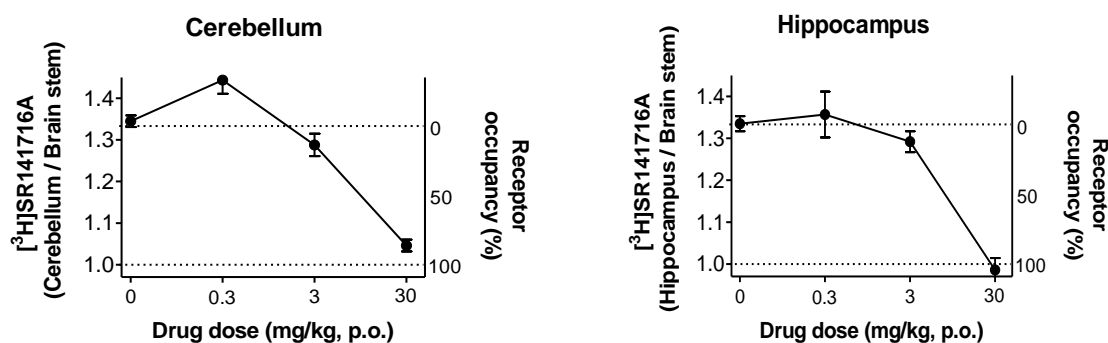


Fig. 2. *In vivo* dopamine transporter occupancy of cocaine and methylphenidate as determined by inhibition of [³H]cocaine binding in the striatum in mice. Values are ratios of radioactivity in striatum (receptor-rich region) to cerebellum (reference region) and are the means of 5 - 6 animals per dose. Both cocaine and methylphenidate reduced specific [³H]cocaine binding with 50% inhibition at about 0.25 mg/kg for both drugs. Dotted line indicates the level of non-specific binding, as determined by administration of a blocking dose of a high-affinity cocaine analogue.

