Dasotraline is a monoamine reuptake inhibitor not a releasing agent as revealed by tetrodotoxin (TTX) sensitivity in the nucleus accumbens of freely-moving rats

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INTRODUCTION
Dasotraline is a novel drug that has been shown in clinical trials to be effective in treating ADHD in adults (Koblan et al, 2015; Hopkins et al, 2016). Dasotraline acts as a potent inhibitor of the human dopamine reuptake transporter (DAT; dopamine uptake IC50 = 3 nM) and noradrenaline (norepinephrine) transporter (NET; noradrenaline uptake IC50 = 4 nM), and a weaker inhibitor of the human serotonin (5-HT) transporter (SERT; serotonin uptake IC50 = 15 nM). Reuptake inhibitors potentiate and prolong the actions of monoamines that have been released by firing-dependent exocytosis, whereas monoamine releasing agents predominantly increase synaptic monoamine concentrations by transport into the nerve terminals and release by displacement of neurotransmitter from the cytosolic and vesicular storage pools (Heal et al, 2013). The sodium channel blocker, tetrodotoxin (TTX), abolishes neuronal firing and is a useful pharmacological tool to differentiate reuptake inhibitors from releasing agents. In this study, we have used microdialysis to study the effects of TTX on dopamine efflux in the nucleus accumbens produced by dasotraline and d-amphetamine.

METHODS
Microdialysis probes (CMA 12 Elite, 2 mm tip) were implanted into the nucleus accumbens (AP +2.2 mm, ML ±1.5 mm relative to bregma, DV −8.0 mm relative to skull surface) of isolurane anaesthetised male, Sprague Dawley rats (300-550g). After ≥16 h recovery, 15 min microdialysate samples (1.2 µM/martil CSF) were collected from freely-moving rats for 2 h after administration of dasotraline (10 mg/kg ip), d-amphetamine (3 mg/kg ip) or vehicle (50 mM acetate buffer, 5 ml/kg ip). TTX (1 µM) was reverse-dialysed into the sampling area via the probe starting 15 min before drug or vehicle injection and it was maintained throughout the experiment.

RESULTS
• Basal efflux of dopamine in the nucleus accumbens was decreased by ≤85% by reverse-dialysis of TTX (1 µM) (Figure 1A). The fall in dopamine efflux was accompanied by marked reductions in the extracellular concentrations of DOPAC (≤58%) and HVA (≤29%) (Figure 1B, 1C).
• Dasotraline (10 mg/kg ip) produced a gradual increase in dopamine efflux that reached a maximum (670 ± 137% above baseline) at 120 min (Figure 1A). Dasotraline evoked a concomitant fall in extracellular DOPAC (≤36%), but not HVA (Figure 2B, 2C).
• Reverse-dialysis of TTX into nucleus accumbens prevented the ability of dasotraline to increase dopamine efflux above vehicle control levels (Figure 1A). Extracellular DOPAC and HVA were reduced compared with the vehicle control in the rats given dasotraline + TTX (Figure 2B, 2C).
• d-Amphetamine (3 mg/kg ip) produced a rapid increase in dopamine efflux in nucleus accumbens with a peak of 2915 ± 634% above baseline at 30 min (Figure 3A). There were concomitant decreases in DOPAC (≤71%) and HVA (≤53%) (Figure 3B, 3C).
• Reverse-dialysis of TTX did not impede the ability of d-amphetamine to increase dopamine efflux in the nucleus accumbens (Figure 3A). Extracellular DOPAC and HVA were reduced compared with the vehicle control in the TTX + d-amphetamine treated rats (Figure 3B, 3C).

CONCLUSIONS
• Dasotraline is a dopamine reuptake inhibitor
• Dasotraline is not a dopamine releasing agent
• Dasotraline’s action is limited to inhibition of reuptake and its mode of action is distinct from that of monoamine releasing agents

REFERENCES