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Selective and interactive effects of D_2 receptor antagonism and positive allosteric mGluR4 modulation on waiting impulsivity

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ABSTRACT

Background: Metabotropic glutamate receptor 4 (mGluR4) and dopamine D_2 receptors are specifically expressed within the indirect pathway neurons of the striato-pallidal-subthalamic pathway. This unique expression profile suggests that mGluR4 and D_2 receptors may play a cooperative role in the regulation and inhibitory control of behaviour. We investigated this possibility by testing the effects of a functionally-characterised positive allosteric mGluR4 modulator, 4-((*E*)-styryl)-pyrimidin-2-ylamine (Cpd11), both alone and in combination with the D_2 receptor antagonist eticlopride, on two distinct forms of impulsivity.

Methods: Rats were trained on the five-choice serial reaction time task (5-CSRTT) of sustained visual attention and segregated according to low, mid, and high levels of motor impulsivity (LI, MI and HI, respectively), with unscreened rats used as an additional control group. A separate group of rats was trained on a delay discounting task (DDT) to assess choice impulsivity.

Results: Systemic administration of Cpd11 dose-dependently increased motor impulsivity and impaired attentional accuracy on the 5-CSRTT in all groups tested. Eticlopride selectively attenuated the increase in impulsivity induced by Cpd11, but not the accompanying attentional impairment, at doses that had no significant effect on behavioural performance when administered alone. Cpd11 also decreased choice impulsivity on the DDT (i.e. increased preference for the large, delayed reward) and decreased locomotor activity.

Conclusions: These findings demonstrate that mGluR4s, in conjunction with D_2 receptors, affect motor- and choice-based measures of impulsivity, and therefore may be novel targets to modulate impulsive behaviour associated with a number of neuropsychiatric syndromes.

1. Introduction

Maladaptive impulsivity, the tendency to act prematurely without foresight, is a multi-faceted behavioural trait associated with impaired response inhibition and a preference for instant gratification (Robbins and Dalley, 2016). Impulsive behaviour is a core feature of attention-deficit/hyperactivity disorder (Castellanos et al., 2006) and drug addiction (de Wit, 2009; Hester and Garavan, 2004; Lee et al., 2009; Moeller et al., 2001), and is thought to manifest from abnormalities in a distributed network of brain regions centred on the prefrontal cortex (PFC), hippocampus, and basal ganglia (Baunez and Robbins, 1997; Dalley et al., 2011; Jentsch and Taylor, 1999; Rieger et al., 2003; Winstanley et al., 2006). Motor and choice impulsivity represent two neurobiologically-dissociable, yet potentially overlapping forms of 'waiting impulsivity' – defined as an intolerance for delayed rewards and an

Abbreviations: cAMP, cyclic adenosine monophosphate; CSF, cerebrospinal fluid; DDT, delay discounting task; EC_{30}, effective concentration at 30%; EC_{50}, effective concentration at 50%; EPSC, excitatory post-synaptic current; 5-CSRTT, five-choice serial reaction time task; GABA, gamma-aminobutyric acid; GP, globus pallidus; HI, high-impulsive; ITI, inter-trial interval; IPSC, inhibitory post-synaptic current; LH, limited hold; LI, low-impulsive; mGluR, metabotropic glutamate receptor; MI, mid-impulsive; MSN, medium spiny neuron; SD, stimulus duration; STN, subthalamic nucleus; TO, timeout.

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inability to refrain from responding during delays signalling future reward (Dalley et al., 2011; Robinson et al., 2009).

Glutamate is the principal excitatory neurotransmitter within the mammalian central nervous system and acts *via* two distinct receptor sub-types; ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors (Conn and Pin, 1997; Schoepp, 2001). Based on distinct neuroanatomical distributions and functional dissociations, as well as the increasing availability of selective allosteric modulators, mGluRs may provide novel targets for therapeutic intervention in a number of neuropsychiatric disorders (Conn and Pin, 1997; Nakanishi, 1992; Schoepp and Conn, 2002). Metabotropic glutamate receptor 4 (mGluR4) is a group III, inhibitory mGluR expressed pre-synaptically within both the ventral and dorsal divisions of the striatum and pallidum (Bradley et al., 1999; Corti et al., 2002), specifically at cortico-striatal glutamatergic and striato-pallidal GABA-ergic synapses (Beurrier et al., 2009; Bradley et al., 1999; Cuomo et al., 2009; Gubellini et al., 2014). Thus mGluR4 is ideally located to modulate the D_2 receptor-expressing indirect pathway (Bradley et al., 1999) projecting from the striatum to the pallidum and subsequently the subthalamic nucleus (STN; Albin et al., 1989; DeLong, 1990; Missale et al., 1998; Smith et al., 1998). Functionally, mGluR4 activation suppresses glutamatergic and GABA-ergic neurotransmission in the striatum and globus pallidus (GP), respectively (Beurrier et al., 2009; Cuomo et al., 2009; Gubellini et al., 2014; Pisani et al., 1997; Valenti et al., 2003).

In the present study, we investigated the effects of positive allosteric mGluR4 modulation on motor and choice impulsivity. Following functional characterisation *in vitro*, we assessed the effects of a selective positive allosteric mGluR4 modulator 4-((*E*)-styryl)-pyrimidin-2-ylamine (Cpd11; East et al., 2010), on premature responding on the 5-CSRTT (Robbins, 2002). To reveal a putative involvement of the D_2 receptors, we investigated the effects of administering the D_2 receptor antagonist eticlopride alone and in combination with Cpd11 on 5-CSRTT performance. We subsequently assessed the effects of sub-chronically administered Cpd11 to reveal possible compensatory effects on different aspects of performance in the 5-CSRTT. For comparative purposes, we also assessed the effects of Cpd11 on choice impulsivity using the delay discounting task (DDT).

2. Material and methods

2.1. Subjects

Male Lister-hooded rats (Charles River, Germany), weighing 250–280 g, were trained and assessed for performance on the 5-CSRTT and DDT. A separate group of Lister-hooded rats, weighing 250–300 g (Charles River, Germany), were used for the assessment of locomotor activity. All rats were housed in groups of four under a 12 h light/dark cycle with food and water initially available *ad libitum*. Food restriction was initiated in the trained rats when body weights were at least 300 g. Animals were provided with environmental enrichment, consisting of red Perspex tunnels and wooden gnawing blocks. Body weight was then maintained at approximately 85% of free feeding weight. All training and testing commenced between the hours of 07:00 and 15:00, five days a week. All experimental procedures were authorised by the Local Animal Care and Use Committee in accordance with local animal care guidelines, AAALAC regulations and the USDA Animal Welfare Act.

2.2. Drugs

Cpd11 was synthesised at Boehringer Ingelheim, Germany. For the functional characterisation experiments conducted *in vitro*, Cpd11 was dissolved in 100% DMSO at a stock concentration of 10 mM and stored at −20 °C. For the *in vivo* studies, all drugs were administered according to a Latin square design unless otherwise stated. Cpd11 was dissolved in 10% Tween80 (0.1% v/v) and 90% Natrosol (0.5%) and administered orally (p.o.) at 2 ml/kg, 30 min before testing. Eticlopride hydrochloride was purchased from Sigma Aldrich (Germany), dissolved in saline (0.9%) and administered subcutaneously (s.c.), at 1 ml/kg, 20 min before testing. *D*-amphetamine was purchased from Sigma Aldrich (Germany), dissolved in saline (0.9%) and administered intraperitoneally (i.p.), 2 ml/kg, 15 min before testing. Drugs that were administered i.p. or s.c. were adjusted to pH 7.4.

The selected dose ranges and pre-treatment times for Cpd11 and eticlopride were based on initial pharmacokinetic studies and preliminary behavioural experiments conducted *in house* (East et al., 2010). For example, 30 min following administration of a 30 mg/kg dose of Cpd11, plasma and CSF concentrations of 11.6 μM and 0.7 μM were measured (CSF:plasma ~0.06). *In vitro,* an EC_{50} of ~1 μM was calculated for Cpd11. Based on these findings, it was necessary to select a dose range sufficient to produce CSF exposures in line with this value. In preliminary behavioural experiments, 30 mg/kg Cpd11 failed to modulate 5-CSRTT performance, indicating a minimum effective dose of approximately 40 mg/kg (based on the estimated CSF:plasma ratio and *in vitro* data). A maximal dose of 80 mg/kg was chosen to ensure high selectivity towards mGluR4; Cpd11 has shown to exert mGluR5 modulation activity at high concentrations (IC_{50} ~ 10 μM) (East et al., 2010). To confirm suitable drug exposures were attained in the behavioural studies, plasma exposures for Cpd11 and eticlopride were assessed using satellite rats (Table 1). CSF exposures for Cpd11 were also assessed and compared to the EC_{50} values calculated *in vitro*.

2.3. Compound characterisation – cAMP assay

The functional and allosteric properties of Cpd11 were assessed *in vitro* using the LANCE® *Ultra* cAMP assay kit (Perkin Elmer, USA) for the determination of changes in intracellular cAMP *via* G_i-coupled receptor modulation. The protocol was based on that provided by Perkin Elmer and is described in detail in the supplementary material (S1).

2.4. Behavioural measures

2.4.1. Five-choice serial reaction time task training

Thirty-two operant chambers (Med Associates Inc, St. Albans, Vermont) were used, as described previously (Bari et al., 2008; Carli et al., 1983). Each chamber consisted of five evenly-spaced apertures containing an LED light, set into a curved wall at the rear of the chamber. A centrally-located food magazine was located on the opposite wall, into which 45 mg reward pellets could be delivered

Table 1
Mean plasma and CSF concentrations of Cpd11 measured 45 min after drug administration (±SEM). Mean plasma concentrations of eticlopride measured 35 min after drug administration (±SEM).

	Mean Plasma Concentration (nM)	Mean CSF Concentration (nM)
Cpd11 (mg/kg; p.o.; n = 4)		
60	17950 ± 2.96	1562.3 ± 299.71
80	20000 ± 1.42	1715 ± 312.89
Eticlopride (mg/kg; s.c.; n = 4)		
0.005	0.78 ± 0.23	–
0.01	1.57 ± 0.83	–
0.02	3.51 ± 0.69	–