Rate dependent effects of SKF 38393 and amphetamine on waiting impulsivity in the 5C-CPT

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Abstract

Rationale ADHD is a neuropsychiatric disorder characterized by inattention and impulsivity. Stimulants such as amphetamine are the most common pharmacological treatment for ADHD. However, such stimulants have substantial abuse and dependence risk. For example, stimulants (smart pills) are commonly used by students to enhance academic performance. Therefore, there is a pressing need for new therapeutic agents. Dysfunction of the dopaminergic system has been associated with ADHD symptoms. D1 receptors play an important role in the cognitive enhancing effects of stimulants and thus may provide a therapeutic target. Previous pre-clinical studies have shown that selective activation of D1 receptors improves sustained attention in the 5C-CPT without improving waiting impulsivity. The present study extends this work to investigate the effect of selective activation of D1 receptors on waiting impulsivity as measured by 5C-CPT.

Objective The aim of the present experiment was to compare the effects of the selective D1 receptor agonist SKF 38393 to a standard ADHD treatment (amphetamine) on waiting impulsivity in the 5C-CPT under extended inter-trial intervals.

Methods Adult female Lister hooded rats were trained to criterion in the 5C-CPT. After rats were able to distinguish target from non-target trials the effects of the selective D1 receptor agonist SKF 38393 (2-6 mg/kg) or amphetamine (0.1-0.4 mg/kg) were investigated under behavioural manipulations to challenge inhibitory response control and, to a lesser extent, attentional resources. The traditional method of analysing drug effects on group data (or sub-groups of high and low performers) was compared with correlational analysis using Oldham method to determine if drug treatment affected waiting impulsivity in a baseline dependent manner.

Results The highest dose of SKF 38393 selectively improved waiting impulsivity in a baseline dependent manner without having a significant effect on sustained attention or response inhibition. The two highest doses of amphetamine improved waiting impulsivity in a baseline dependent manner without having a significant effect on sustained attention and response inhibition.

Conclusions The effects of AMPH on waiting impulsivity further supports the predictive validity of 5C-CPT. In addition, this study taken together with previous studies which report pro attentional effects of D1 receptor agonists suggests that D1 selective agonists could be therapeutically beneficial in ADHD patients. Clinical trials are needed to confirm these effects. This is the first study to take advantage of the variation in individual baseline waiting impulsivity to demonstrate the presence of a rate-dependent relationship between baseline waiting impulsivity and magnitude of improvement following stimulant or non-stimulant medications. Oldham's correlation method may present an opportunity to enhance the translational value of research in the preclinical laboratory to the clinic. Furthermore, these findings further support the ability of 5C-CPT to distinguish between response inhibition and waiting impulsivity and allow symptom specific treatment.

Introduction

Attention deficit hyperactivity disorder (ADHD) is a psychiatric disorder characterized by three main behavioural deficits: lack of attention, hyperactivity and impulsivity (Mueller et al., 2017). These are observed during early childhood and often persist into adult life. The prevalence of ADHD is between 2-7%: 5% of children have difficulties with inattention and hyperactivity and 2-4% of adults are diagnosed with ADHD (Sayal et al., 2018). With increasing age, inattention becomes more prominent while impulsivity diminishes (Faraone et al., 2015; Telzer et al., 2016). Children diagnosed with ADHD have a higher risk of developing social and cognitive problems in later life (Lara et al., 2009). Depending on the main symptoms, ADHD can be divided into three categories: lack of attention (most prevalent among girls), hyperactivity (more prevalent among boys) and a third subtype that is a combination of the first two (Rasmussen and Gillberg, 2000). ADHD is more common in boys than in girls. However, ADHD is unrecognised and underdiagnosed in many countries. In the UK less than 50% of children with inattention and hyperactivity have been diagnosed with ADHD (Faraone et al., 2015). Stimulants such as methylphenidate and amphetamine are the most common pharmacological treatment for ADHD (Mueller et al., 2017).). However, stimulants have the potential to lead to abuse and dependence (Ahn et al., 2013). For example, stimulants (smart pills) are commonly used by students to enhance academic performance (Sharma et al., 2014). Non-stimulants are the second line treatment for ADHD; however, they are less effective than stimulants and they have a delayed onset of action. Therefore, there is need for new therapeutic agents without the dependence potential (Sharma et al., 2014). One of the characteristic features of ADHD is impulsivity (behavioural disinhibition), which is important to minimise in order to suppress inappropriate action (Arnsten, 2009). Behavioural disinhibition may lead to actions being carried out rapidly, without thinking of their consequences, or it may lead to compulsivity, which refers to repetitive and persistent performance of a certain action even though it does not lead to a reward (Dalley et al., 2011; Faraone et al., 2015). Deficits in four subtypes of impulsivity have been reported in ADHD: (a) response disinhibition (impulsive action), which involves difficulty in inhibiting a pre-potent response; (b) poor waiting impulsivity, which is seen as difficulty in waiting for stimulus presentation before responding; (c) temporal discounting (decisional impulsivity or impulsive choice), which involves preference for immediate reinforcement over delayed; and (d) poor stopping impulsivity (action cancellation), which manifests as a difficulty in stopping an ongoing response (Dalley et al., 2011). The medial prefrontal cortex and ventral striatum (particularly the nucleus accumbens core (NAc) have been implicated in waiting impulsivity, usually measured in rodents using the 5-CSRTT, which measures the number of premature responses made before stimulus presentation (Dalley et al., 2011; Christakou et al., 2004; Caprioli et al., 2014). Lesions of the medial prefrontal cortex and nucleus accumbens core have been associated with impulsive behaviours in the form of increased premature response in 5-CSRTT (Chudasama et al., 2003; Christakou et al., 2004). Subsequent studies used the 5C-CPT paradigm to examine the effects of pharmacological manipulation of the dopaminergic system on cognitive functions, including sustained attention and impulsivity. The mesolimbic dopaminergic system has been associated with inhibitory response control (Granon et al., 2000; Dalley and Robbins, 2017). D1 receptors are mainly expressed in PFC and evidence suggests that activation of specific dopamine receptor subtypes (particularly D1 and D4) can alter behaviour measures associated with 5C-CPT performance. For example, administration of a D1 receptor agonist (SKF-38393) enhanced vigilance by increasing hit rate without affecting response inhibition (the probability of false alarm) under challenging conditions (extended ITI) in rats (Barnes et al., 2012), suggesting that selective activation of D1 receptors represent a promising approach to the treatment of ADHD. However, this drug impaired performance under standard task conditions, suggesting that the effectiveness of D1 receptor agonists in enhancing attention depends on basal dopamine levels (Barnes et al., 2012). Thus, this study suggests that, under high task demands, activation of D1 receptors improves certain domains of cognitive function (such as attention) without affecting other domains such as impulsivity and response inhibition

(Barnes et al., 2012). Despite these findings, the role of D1 receptor agonists on waiting impulsivity under extended inter-trial interval is yet to be determined. It has been reported that activation of the dopaminergic system improves performance in dopaminergically-compromised subjects but impairs performance in normal subjects, suggesting that the relationship between attentional performance or inhibitory response control and dopamine level follows an inverted-U shaped curve (Tomlinson et al., 2015; Hayward et al., 2016; Bickle et al., 2016). These results indicate that increasing dopamine neurotransmission under high task demands by activation of specific receptor subtypes normalises the dopaminergic deficit in low performing rats; however, further activation of a normal dopaminergic system or in subjects with an elevated basal dopamine level (such as high performers) will lead to excessive release of dopamine, which will impair performance (Tomlinson et al., 2014; Hayward et al., 2016). Although there is evidence to support the baseline-dependent effect of stimulants on 5-CSRTT or 5C-CPT performance, few studies have used correlational analysis to explicitly test this.

Clinical and preclinical findings support the role of the dopaminergic system in attention and response inhibition, however, the exact mechanisms by which drugs that increase activity of the dopaminergic system improve cognitive function in individuals with ADHD is poorly understood (Arnsten, 2009; Mueller et al., 2017; Faraone et al., 2015). Understanding the mechanism of action of these drugs will facilitate drug discovery efforts for ADHD. The goal of the present study was to examine the role of amphetamine and D1 receptor agonists on inhibitory response control as assessed by 5C-CPT performance. Amphetamine (AMPH) was used to test the predictive validity of 5C-CPT for assessing pharmacologically induced enhancement in waiting impulsivity. Previous studies have reported no change in inhibitory response control following administration of SKF-38393 under task conditions that target attention and, to a lesser extent, impulsivity (Barnes et al., 2012). Therefore, we used an extended inter trial interval (ITI) and an extended session length challenge to determine whether SKF 38393 can alter waiting impulsivity under challenging task conditions that target inhibitory response control. Correlation analysis have been used previously to determine the rate-dependent effect of pharmacological manipulation of dopaminergic system on inhibitory response control (Bickle et al., 2016). We, therefore, applied correlational analysis in this study to determine if SKF 38393 or amphetamine can modulate waiting impulsivity in a baseline-dependent manner.

Materials and Methods

Animals

Female Lister hooded rats (n=15; Charles River, UK) were housed in groups of 5 on a reversed 12h light cycle in a temperature (21 ± 2 °C) and humidity (55±5%) controlled environment (University of Manchester BSF facility). Rats weighed 230±10g upon arrival. They were allowed free access to food (Special Diet Services, UK) and water for one week prior to the beginning of training. Home cages were individually ventilated cages with two levels (GR1800 Double-Decker Cage, Techniplast, UK) and testing was completed under a standard 12-hour light: dark cycle (lights on at 7:00 am). Two days before training commenced food restriction was initiated to encourage their task engagement and this continued throughout training. Rats were maintained at approximately 90% of their free-feeding body weight (fed 10 g rat chow/rat/day) and were provided with free access to water. All procedures were conducted in accordance with the UK animals (Scientific Procedures) 1986 Act and local University ethical guidelines.

Rat 5C-CPT Apparatus

Training took place in 8 operant chambers (Campden instruments Ltd, UK), each placed inside a ventilated and sound-attenuating box. The chambers were of aluminium construction 25cm × 25 cm. Each chamber had a curved wall containing nine apertures. For 5C-CPT training, four of the apertures were blocked by metal caps while the other five were left open (numbers 1, 3, 5, 7 and 9 were open). Each aperture had a light and an infrared beam crossing its entrance to record beam breaks following nose poke. A reward dispenser was located outside each chamber that automatically dispensed reinforcers (45 mg sucrose food pellets; Rodent Pellet, Sandown Scientific, UK) into a food tray at the front of the chamber. Entrance to each food tray was covered by a hinged panel so that tray entries were recorded when the rat's snout opened the panel. House lights were placed in the side wall of the chambers and performance was monitored by a camera attached to the roof.

5C-CPT behavioural training

The 5C-CPT was modified from the 5-CSRTT to incorporate non-target trials in which all five holes are lit and the rat must not respond at any of the five positions to be rewarded (Young et al., 2009; Tomlinson et al., 2014). The incorporation of non-target trials helps to dissociate response inhibition from waiting impulsivity; premature responses during target trials reflect a deficit in waiting impulsivity, while the failure to withhold responding (false alarms) during non-target trials represents response disinhibition (Young et al., 2011). This task also allows the assessment of vigilance in rodents in a manner consistent with human CPT using SDT (Young et al., 2009). In addition, a variable ITI is incorporated in 5C-CPT to prevent rats from timing their response (Van Enkhuizen et al., 2014). 5C-CPT training was carried out 5 days per week. A session consisted of 120 trials or was 30 min long, whichever came first. The training consisted of 3 phases**:** (i) 5-CSRTT training, (ii) 5C-CPT training with fixed ITT and (iii) 5C-CPT training with variable ITI (Tomlinson et al., 2014; 2015). The task parameters such as stimulus duration and limited hold were gradually reduced for rats based on their individual performance. Some of the key parameters that are used to assess performance in the task are listed in Table 1.

Table 1: Description of 5-CPT outcome measures.

Outcome measures	**Descriptions**	**Dimension**
Correct response (Hit)	Response made in the illuminated hole	
Incorrect response	Response made but in the wrong hole	
Error of Omission (Miss)	Failure to respond to the stimulus within a certain period.	
Accuracy	Proportion of hits to incorrect responses	Selective attention
Correct rejection	Correctly withholding the response to non-target stimulus	
False alarm	Failure to withhold the response to non-target stimulus	
Hit rate (HR)	Proportion of 'hits' to 'misses'	
False alarm rate (FAR)	Proportion of false alarms to correct rejections	Response inhibition
Discriminability index (*d*')	Ability of rats to distinguish between target and non-target trials	Sustained Attention
Responsivity index (Bias)	Tendency to respond to stimuli	Motivation
Premature response	Response made before stimulus presentation	Waiting impulsivity

Perseverative response	Repetitive responses (repeated nose pokes) even after making a response	Compulsive behaviour
Correct latency	Time taken to make a correct response	Attention
Correct magazine	Time taken to collect the reinforcer after the correct response is made	Motivation

Signal detection theory (SDT)

Two primary parameters are calculated from 5C-CPT trial-outcome measures. The first is hit rate, which is the proportion of 'hits' to 'misses' and the second is false alarm rate, which is the proportion of false alarms to correct rejections (Young et al., 2011; Table 2). From these two parameters, sensitivity (vigilance) and bias can be calculated using SDT (Young et al., 2011). Sensitivity reflects the ability to respond to the illuminated hole and inhibit that prepotent response when all five holes are illuminated. Sensitivity can be estimated by calculating the discriminability index (*d'*), which is the difference between z score of hit rate and false alarm rate (Young et al., 2009; Anderson, 2015). 5C-CPT performance can also be affected by bias (tendency to respond to stimuli), which can be measured non-parametrically by calculating the responsivity index (RI). A positive value for RI reflects bias towards FA (liberal response strategy), while a negative RI reflects bias towards omission (conservative response strategy). When RI is zero there is no bias (Anderson, 2015; Young et al., 2013).

Table 2 Parameters generated form 5C-CPT trials. Hit = correct response, Miss = omission, CR = correct rejection, FA = false alarm, FAR = false alarm rate, HR = hit rate, *d'* = discriminability index and RI = responsivity index.

Trial Type	Measure	Calculations	SDT parameters
Target trials	Hit and Miss	$HR = \frac{Hit}{Hit + Miss}$	$d' = Z(HR) - Z(FAR)$
Non-target trials	CR and FA	$FAR = \frac{FA}{FA + CR}$	$RI = \frac{HR + FAR - 1}{1 - (FAR - HR)^2}$

Experimental Design

Drug preparation

d-Amphetamine sulphate (Sigma, UK) was dissolved in 0.9 % saline. SKF 38393 (Sigma, UK) was dissolved in distilled H_2O. Both drugs were injected via the intraperitoneal (i.p.) route at a volume of 1 ml/kg. All drug doses were calculated as base equivalent weight. The doses used in this study were chosen based on previous studies performed in rats (Barnes et al., 2012). AMPH doses were selected to be lower than those that increase locomotor activity (1.25 mg/kg) (Turner et al., 2015; 2017).

Behavioural testing - assessing effects of SKF 38393on waiting impulsivity.
All rats had been exposed previously to PD128907. After a two-week washout period, SKF 38393 was administered. Fifteen rats from the first cohort were used to investigate the effect of SKF 38393 on 5C-CPT performance using challenging task conditions that consisted of a prolonged ITI (12,14 and 16s) in a one-hour session using a within-subjects design. Following task acquisition rats received 2, 4 or 6 mg/kg SKF-38393 30 min before testing.

Assessing effects of Amphetamine on waiting impulsivity. Fifteen rats were used to investigate the effect of amphetamine on 5C-CPT performance using challenging task conditions that consisted of a prolonged ITI (8,10 and 12s) in a one-hour session. Using a within-subjects design trained rats received 0.1, 0.2 or 0.4 mg/kg amphetamine or vehicle, 10 min before the task.

Statistical analysis

All data were displayed as observed mean ± SEM. Overall performance was analysed by one-way repeated measures ANOVA followed by Planned Comparisons. Analysis of the performance across the duration of the session was carried out by two-way repeated measures ANOVA (Trial and treatment as within-subject factors) followed by Planned Comparison on the predicted means. If the outcome of the repeated-measures ANOVA yielded significant effects of dose, further post-hoc analysis was performed using Dunnett multiple comparison test. Analysis of the performance across the vITI was carried out by two-way repeated measures ANOVA (ITI and Treatment as within-subject factors), followed by Planned Comparisons. Alpha level was set to 0.05 and all analyses were carried out using Prism (v8.0).

Correlational analysis

Three methods are used in literature to assess the relationship between baseline and magnitude of improvement following drug administration (Table 3). This study investigated if baseline performance predicted the degree of change in performance following administration of GBR using Oldham method. Oldham's method was used to determine the presence of a rate dependent relationship. Pearson correlation was used because individuals were normally distributed. Oldham's $r > 0.3$ indicates the presence of a rate dependent relationship (Oldham, 1962; Snider et al., 2016).

Table 3 Three methods are used in literature to assess the relationship between baseline and magnitude of improvement following drug administration. (Oldham, 1962; Bickel et al., 2016; Turner et al., 2017; Snider et al.,2018).

Method	Descriptions	
Simple method	Correlation between baseline values and the drug induced change (Spurious correlation)	This method is affected by mathematical coupling and regression to the mean
Oldham's method	Correlation between average of pre- and post-treatment values and the drug induced change	Eliminates the effect of mathematical coupling and regression to the mean
Re-test method	Correlation between adjusted baseline value and the drug induced change	Reduces the bias associated with regression to the mean

Regression to the mean is detected when repeated measurements are taken from the same subject. For example, if baseline premature response after vehicle administration is high, the probability of having a lower premature response after drug administration is greater than having a higher premature response and vice versa. Regression to the mean can also happen at sub-group levels (Chiolero et al., 2013). Mathematical coupling occurs during correlations where one variable is part of the calculation for the other variable, resulting in a spurious

correlation (Tu et al., 2004). This false correlation will be present irrespective of any true relationship between the two measurements. In simple correlation, the baseline value is part of calculation of the change following drug administration (drug value – baseline value); therefore, these values are not independent as there is a mathematical coupling between them (Tu et al., 2004; Chiolero et al., 2013). This coupling induces a strong negative false correlation when baseline value is plotted against the change following drug administration. To avoid the biases associated with the simple method, one may assess the relationship between the average of pre- and post-treatment value with the change following drug administration (Oldham's method). Here, Oldham suggested that the average is closer to the true baseline value than the single value obtained following vehicle administration (Oldham, 1962). In addition, the average is not part of calculation of the change (Tu et al., 2004; Chiolero et al., 2013). This method has been used in literature to determine the presence of a rate-dependent effect (e.g., Bickel et al., 2016; Snider et al., 2016; Snider et al., 2018).

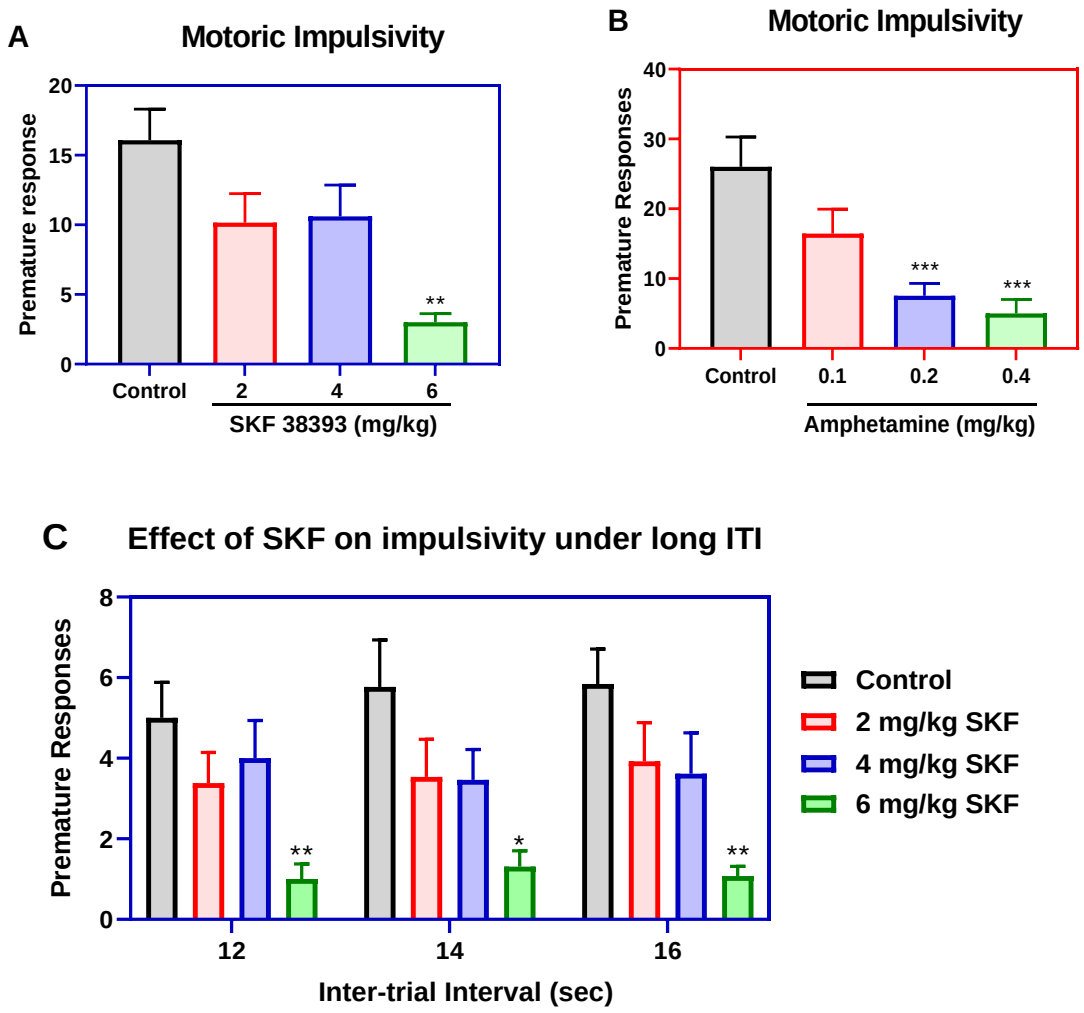
Results

waiting impulsivity

Group performance. Effects of SKF-8393 or amphetamine (AMPH) on motoric impulsivity is shown in Fig.1. SKF-38393 produced a significant decrease in premature responding on the 5C-CPT (F (2.631- 31.58) = 6.688, $p < 0.01$; Fig.1A). Dunnett *post hoc* test revealed a significant decrease in premature responding between vehicle and 6 mg/kg SKF-38393 treated rats ($p<0.01$). Amphetamine produced a significant decrease in premature responding in the 5C-CPT (F (1.948, 23.38) = 12.80, $p < 0.001$; Fig.1B). Dunnett *post hoc* test revealed a significant decrease in premature responding following 0.2 or 0.4 mg/kg compared with control ($p< 0.001$). Effects of SKF 38393 on motoric impulsivity across different ITIs is shown in Fig. 1C; a two-way repeated measures ANOVA revealed a significant treatment effect [F (2.482, 29.78) = 9.686 $p < 0.001$] on impulsivity with no significant ITI effect [F (1.561, 18.73) = 0.1718, P=0.7906] and no interaction [F (2.673, 32.08) = 0.2205, P=0.8610]. Baseline impulsivity remained stable over all inter-trial intervals. Dunnett's multiple comparison *post hoc* test revealed that 6 mg/kg SKF 38393 significantly reduced the number of premature responses at 12s ($p< 0.01$), 14s ($p< 0.05$) and 16s ($p< 0.01$) ITIs, indicating that the effect of SKF 38393 was consistent across all intervals. Effects of amphetamine on motoric impulsivity over the different inter-trial intervals is shown in Fig. 1D, where a two-way repeated measures ANOVA revealed a significant treatment effect (F [F (1.970, 23.64) = 12.63, $p < 0.001$) on impulsivity with a significant ITI effect [F (1.286, 15.43) = 9.987, $p < 0.01$] and no interaction [F (2.234, 26.81) = 2.341, P=0.1105]. Baseline impulsivity was significantly higher during 12s compared with 8s ITI. Dunnett's multiple comparison test revealed that 0.2 mg/kg amphetamine significantly reduced the number of premature responses at 8s, 10s and 12s ($p< 0.05$, $p< 0.05$ and $p< 0.001$, respectively) inter-trial intervals, indicating that effect of amphetamine was consistent across all intervals. Dunnett's multiple comparison test also revealed that 0.4 mg/kg amphetamine significantly reduced motoric impulsivity at 8s ($p< 0.01$), 10s ($p< 0.01$) and 12s ($p< 0.001$) inter-trial intervals, indicating that effect of amphetamine was consistent across all inter-trial intervals durations. Effects of SKF on impulsivity across the session is shown in Fig. 1E, where each session consisted of 163 trials broken into 4 blocks, each block consisting of 40 trials. A two-way repeated measures ANOVA revealed a significant trial effect [F (2.190, 26.28) = 11.26, $p < 0.001$] and treatment effect [F (2.628, 31.54) = 6.570, $p < 0.05$] on impulsivity and a significant interaction [F (4.690, 48.99) = 2.917, $p < 0.05$]. Dunnett *post hoc* tests revealed a significant decrease in the number of baseline premature responses in trial block 4 compared with trial block 1 ($p< 0.01$), indicative of improvement in waiting impulsivity

over time. Dunnett tests also revealed that the highest dose of SKF 38393 reduced waiting impulsivity in the first, second and third ($p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively) trial blocks. However, 2mg/kg and 4 mg/kg SKF 38393 reduced waiting impulsivity only in the first trial block (all $p < 0.05$) when compared to saline treated rats. Effects of amphetamine across the session is shown in Fig.1F, where sessions consisted of 163 trials broken into 4 blocks, with each block consisting of 40 trials. A two-way repeated measures ANOVA approach revealed significant trial [$F(1.888, 22.66) = 15.72$, $p < 0.0001$] and treatment effects [$F(1.913, 22.96) = 12.82$, $p < 0.001$] on impulsivity and a significant trial period x treatment interaction [$F(3.546, 42.55) = 2.748$, $p < 0.05$]. Dunnett tests revealed a significant decrease in the number of baseline premature responses in trial block 4 (123-163) compared with trial block 1 (0-40; $p < 0.01$), indicative of improvement in waiting impulsivity over time. Dunnett tests also showed that 0.2 mg/kg and 0.4 mg/kg amphetamine reduced waiting impulsivity in all trial periods.

Waiting impulsivity



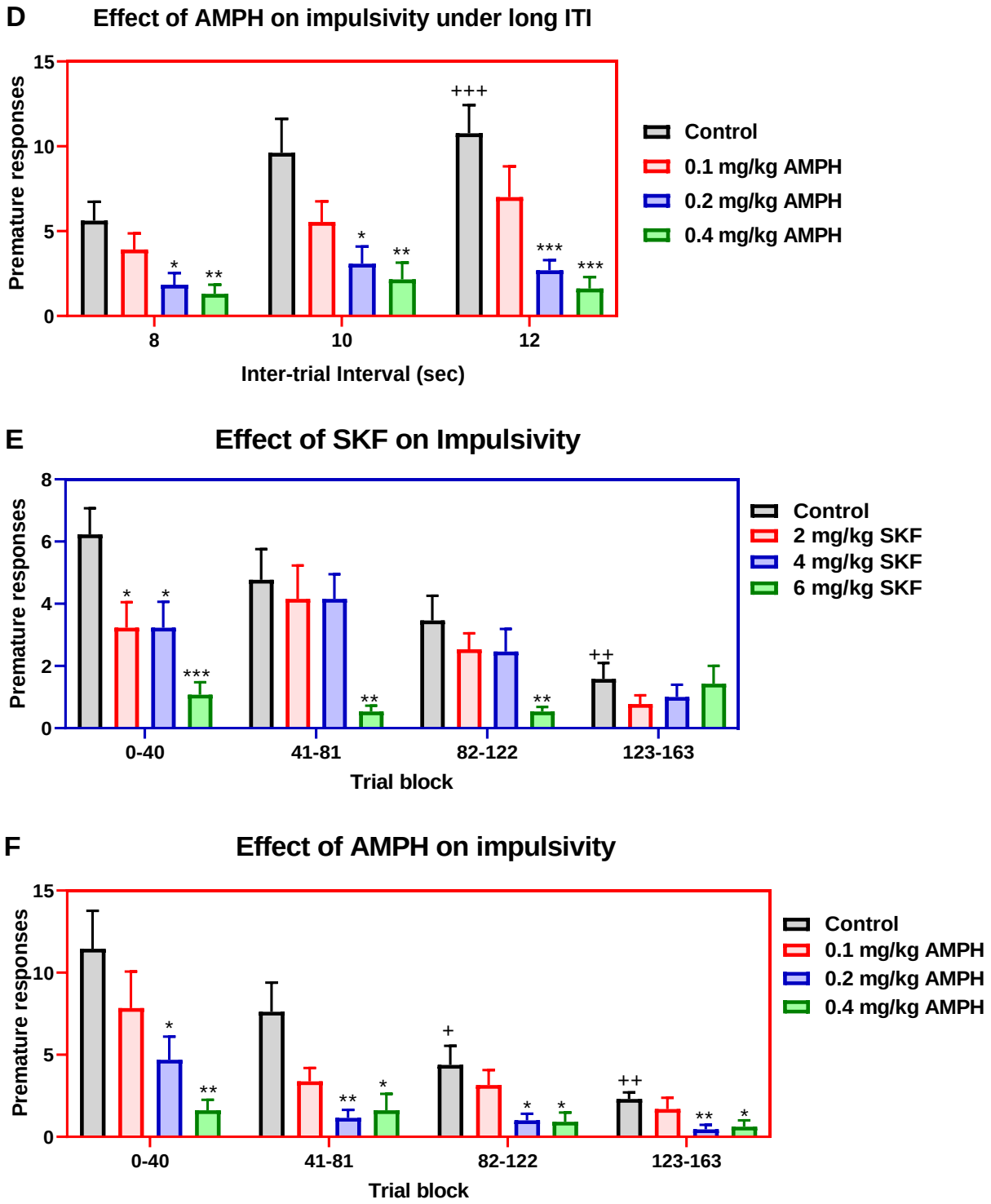


Figure 1: Differential effect of SKF 38393 (2, 4 or 6 mg/kg) or amphetamine (0.1, 0.2 or 0.4 mg/kg) on waiting impulsivity of rats tested under long inter-trial intervals (n=15). (A and B) Impulsivity averaged over all ITIs. (C and D) Impulsivity over the three different inter-trial intervals. (E and F) Impulsivity over session trials, where each session consisted of 163 trials broken into 4 blocks, each block consisting of 40 trials. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to vehicle-treated rats (Control) of the same ITI or trial block (Dunnett). + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, ++++ $p < 0.0001$ compared to control of the first block or the shortest ITI (Dunnett).

Correlational analysis. Relationship between baseline impulsivity and SKF or AMPH induced change using Oldham method is shown in Fig 2. Oldham method revealed a significant strong negative correlation between baseline premature response and the SKF-induced change at the highest dose, consistent with rate-dependence (4.2A). Oldham method revealed strong negative correlations between baseline premature response and the change following administration of 0.2 mg/kg amphetamine (r = -0.78, $p < 0.01$) and 0.4 mg/kg amphetamine (r = -0.65, $p < 0.05$), consistent with rate-dependence (4.2B). The most impulsive rats showed greater decrease in premature responding following amphetamine administration.

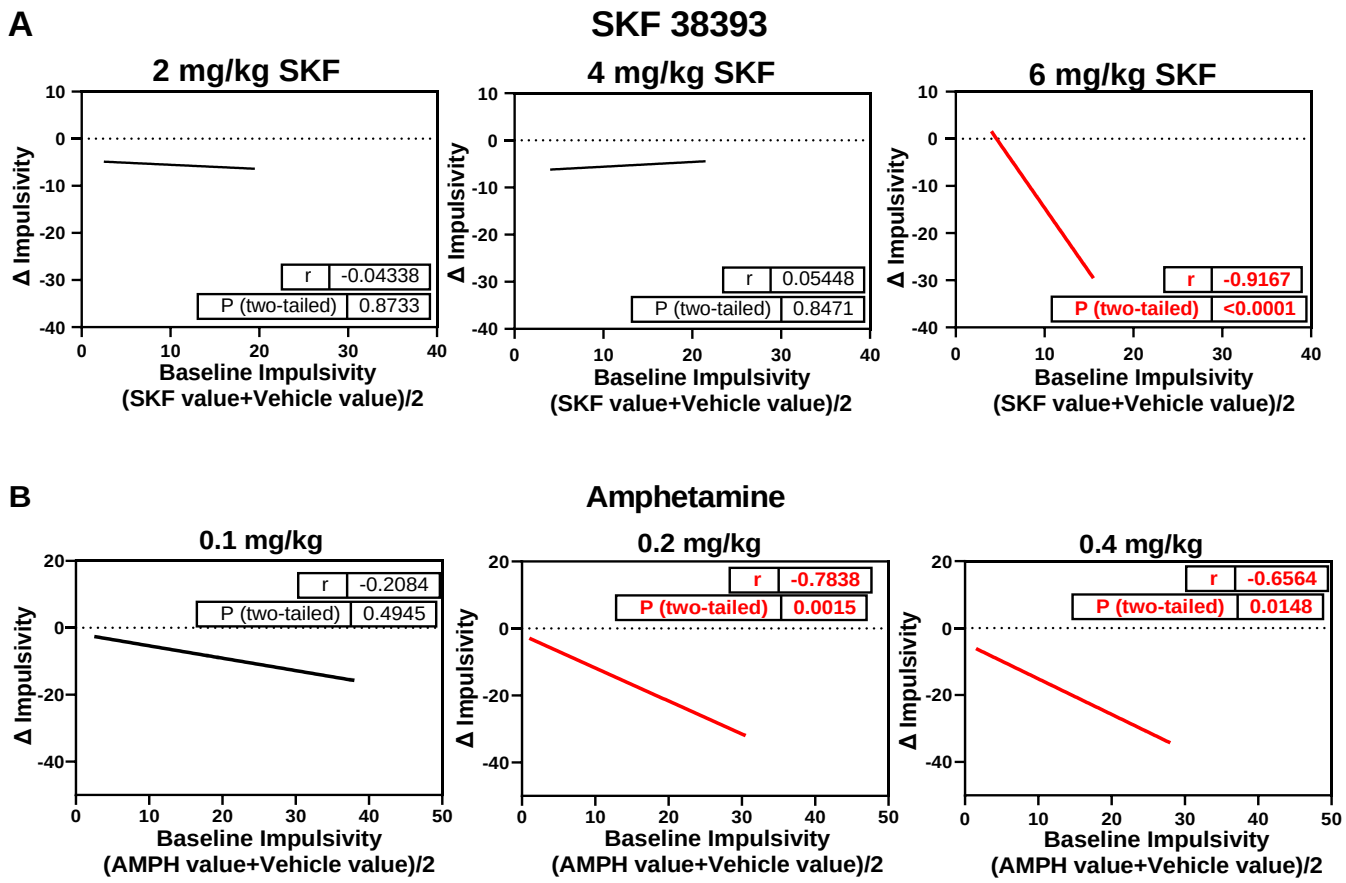


Figure 2: Relationship between baseline impulsivity and SKF 38393 (2, 4 or 6 mg/kg) or amphetamine (0.1, 0.2 or 0.4 mg/kg) induced change using Oldham's method (n=15). (A) Oldham's correlation between baseline impulsivity and SKF induced change. (B) Oldham's correlation between baseline impulsivity and AMPH induced change. The red regression lines represent statistically the significant relationships.

There was wide variability in baseline premature response under each inter-trial interval. Therefore, the individual baseline premature responses of rats were plotted against magnitude of improvement following administration of SKF 38393 or amphetamine under each ITI. The highest dose of SKF changed impulsivity in a baseline dependent manner at all the ITI. The two highest doses of AMPH improved waiting impulsivity in a rate dependent manner at 10s and 12s ITI (see Supplement).

Responsivity index (Motivation).

Effects of SKF on responsivity index is shown in Fig 3A two-way repeated measures ANOVA revealed no significant trial effect [F (1.430, 17.17) = 0.5885, p = 0.5116] and a significant treatment effect [F (2.628, 31.54) = 6.570, $p < 0.05$] on responsivity index and no significant interaction [F (5.153, 52.67) = 3.333, p = 0.6538]. There was no significant change in baseline responsivity index (motivation) across the session. Dunnett tests also revealed that the intermediate dose of SKF 38393 increased responsivity in the first and third ($p < 0.05$ and $p < 0.01$ respectively) trial blocks. Effects of amphetamine on responsivity index is shown in Fig 3B. A two-way repeated measures ANOVA revealed no significant trial effect [F (2.392, 31.09) = 1.713, p =0.192] and no significant treatment effect [F (1.695, 22.03) = 0.6690, p = 0.498] on responsivity index and no significant interaction [F (3.429, 41.52) = 1.726, p = 0.17]. Together, these findings suggest that the decrease in baseline waiting impulsivity at the end of the session was mainly due to improvement in inhibitory response control.

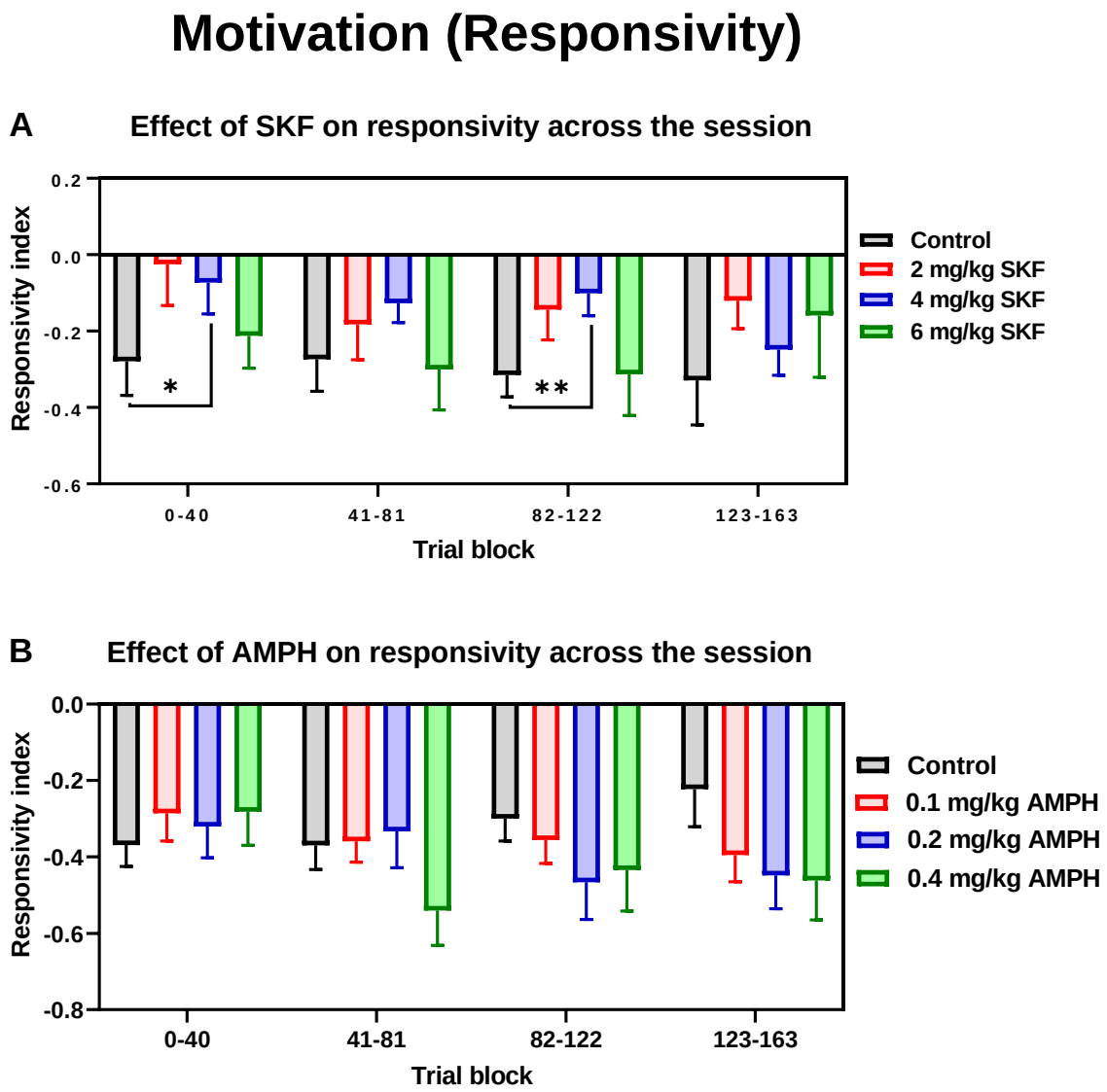


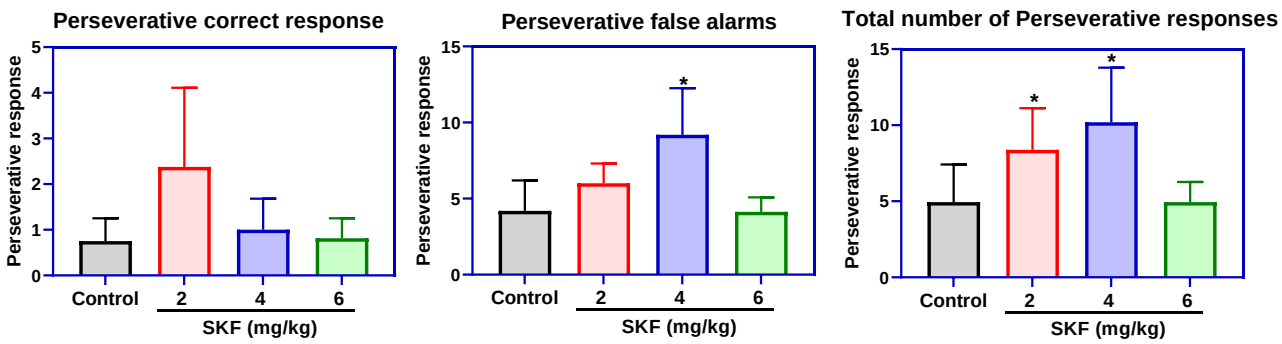
Figure 3. Effects of SKF 38393 (vehicle, 2, 4 or 6 mg/kg) or amphetamine (vehicle, 0.1, 0.2 or 0.4 mg/kg) on responsivity index over session trials (n= 15), where each session consisted of 163 trials broken into 4 blocks, each block consisting of 40 trials. * $p < 0.05$ and ** $p < 0.01$ compared to vehicle treated rats (Control) of the same block (Dunnett).

Compulsive behaviour

Effects of SKF 38393 on compulsive behaviour is shown in Fig. 4A. A one-way repeated measures ANOVA revealed insignificant treatment effect [F (1.063, 15.94) = 1.495, P=0.2413] on perseverative correct response and a significant treatment effect [F (1.719, 25.79) = 3.617, $p < 0.05$] on perseverative false alarm. Dunnett's multiple comparison *post hoc* test revealed a significant increase in perseverative false alarm ($p < 0.05$) at 4mg/kg SKF 38393 when compared to control. In addition, one-way repeated measures ANOVA revealed a significant treatment effect [F (1.837, 27.56) = 4.358, $p < 0.05$] on total number of perseverative responses. Dunnett's multiple comparison *post hoc* test revealed a significant increase in total number of perseverative responses ($p < 0.05$) at 2 mg/kg or 4mg/kg SKF 38393 when compared to control. Effects of amphetamine on compulsive behaviour is shown in Fig 4B. A one-way repeated measures ANOVA revealed insignificant treatment effect [F (1.250, 16.25) = 0.4516, $p = 0.55$] on perseverative correct response or on perseverative false alarm [F (2.513, 32.67) = 1.628, $p = 0.2$]. In addition, one-way repeated measures ANOVA revealed insignificant treatment effect on total number of perseverative responses [F (1.375, 17.88) = 0.1763, $p = 0.7575$]. The relationship between baseline perseverative response and SKF 38393 or amphetamine induced change is shown in Fig. 4 (C and D). Oldham method revealed a positive correlation between baseline total number of perseverative responses and the change following administration of 2 mg/kg or 4 mg/kg SKF 38393 (Fig.4C), indicative of a rate-dependent effect. This finding suggests that the two lowest doses of SKF 38393 impaired compulsive behaviour in baseline dependent manner. On the other hand, Oldham method revealed no relationship between baseline perseverative responses and magnitude of change following administration of amphetamine (Fig.4D).

Compulsive behaviour

A **Effects of SKF on Compulsive behaviour**



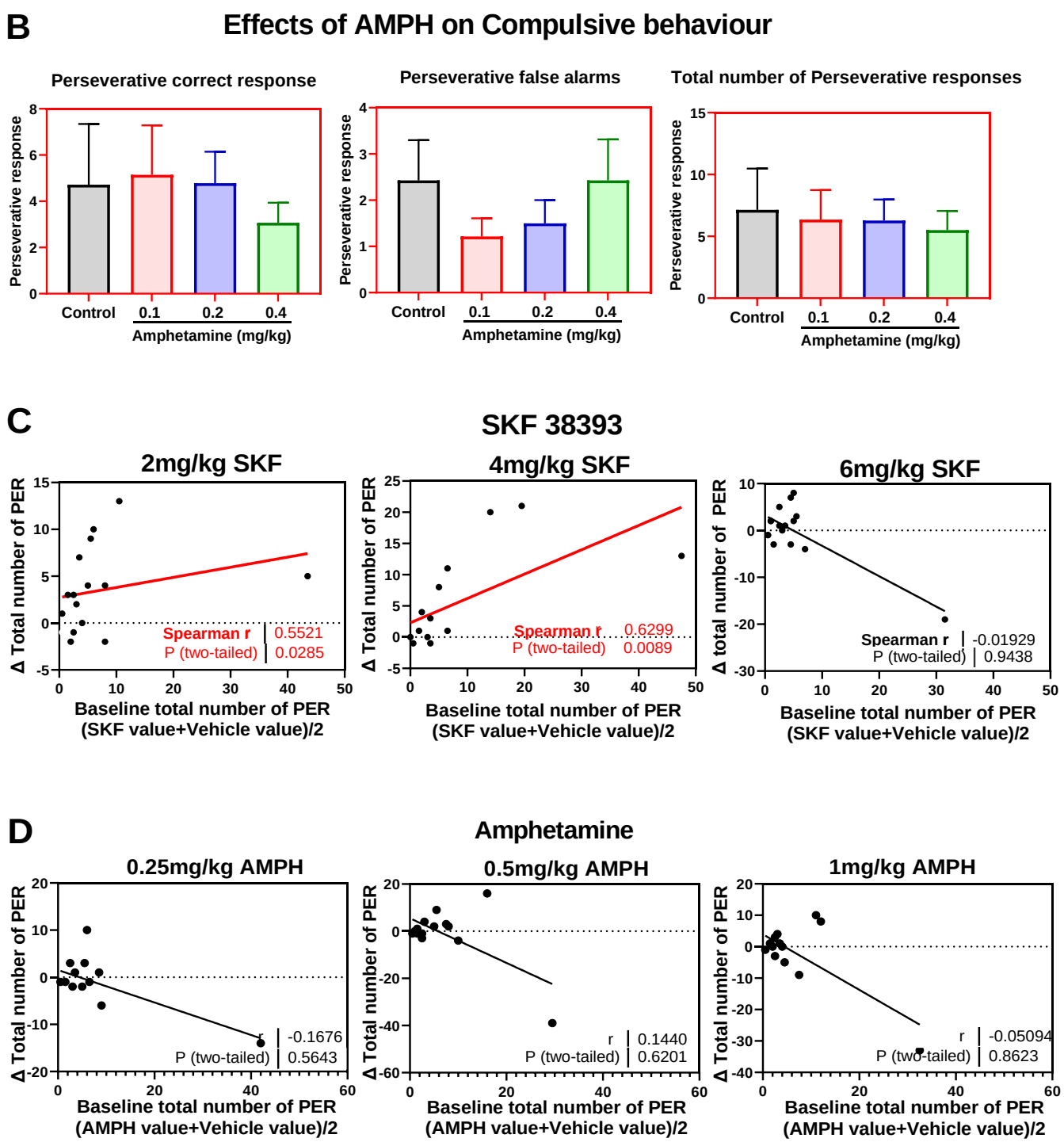


Figure 4: Effects of SKF 38393 (2, 4 or 6 mg/kg) or amphetamine (0.1, 0.2 or 0.4 mg/kg) on compulsive behaviour. (A) Effects of SKF on compulsive behaviour. (B) Effects of AMPH on compulsive behaviour. (C and D) Relationship between baseline compulsivity (total number of perseverative responses) and magnitude of change following administration of SKF 38393 or amphetamine using Oldham method. *$p < 0.05$ compared to vehicle-treated rats (Dunnett). The red regression lines represent statistically significant relationships. PER = perseverative responses.

Other 5C-CPT performance measures

Effects of SKF on other 5C-CPT performance measures are shown in Supplement Figure 1A-C. One-way repeated measures ANOVA revealed no significant treatment effect on accuracy [F (2.872, 34.47) = 0.5714, P=0.6304], d prime [F (2.281, 27.37) = 2.148, P=0.1304] and false alarm rate [F (2.653, 31.84) = 1.003, P=0.3968]. One-way repeated measures ANOVA revealed a significant treatment effect on omission [F (2.297, 27.56) = 6.072, $p < 0.05$], responsivity index [F (2.304, 27.64) = 4.471, $p < 0.05$] and hit rate [F (2.344, 28.13) = 3.735, $p < 0.05$]. Dunnett's post hoc test revealed a significant decrease in omission ($p < 0.05$) at 2 mg/kg SKF 38393 when compared to control. In addition, Dunnett's post hoc test revealed a significant increase in responsivity at 2 and 4 mg/kg compared with vehicle (control) (all $p < 0.05$). Furthermore, Dunnett's post hoc test revealed a significant increase in hit rate at 4 mg/kg compared with vehicle control (all $p < 0.05$). Effects of AMPH on other 5C-CPT performance measures are shown in Supplement Figure 1D-F.One-way repeated measures ANOVA revealed no significant change in accuracy [F (2.364, 28.37) = 0.1872, P=0.8633], omission [F (1.972, 23.66) = 5.276, P=0.056], d prime [F (2.222, 25.18) = 3.217, P= 0.0524], responsivity index [F (2.298, 27.58) = 0.9271, P=0.4194] , hit rate [F (2.077, 24.93) = 5.109, P= 0.07] and FA rate [F (2.072, 24.86) = 0.1214, P=0.8925] following administration of AMPH.

Discussion

D1 receptors have long been implicated in attentional processes and inhibitory response control as measured by the 5-CSRTT and 5C-CPT (Barnes et al., 2012; Zhu et al., 2017). Previous studies suggest that the ability of D1 receptor agonists to improve attentional performance in the 5-CSRTT depends on baseline accuracy performance. For example, systemic administration of SKF-38393 impaired accuracy of rats with high baseline accuracy (Zhu et al., 2017), while direct infusion into the striatum or medial prefrontal cortex increased accuracy of rats with low baseline accuracy (Granon et al., 2000). The work in this thesis extends previous findings by investigating the effects of D1 receptor agonist on waiting impulsivity in the 5C-CPT under challenging task conditions. Our findings show that the highest dose of SKF-38393 under high task demand (prolonged ITI) improved waiting impulsivity in a rate-dependent manner. This finding is consistent with pro-attentional effects of 6 mg/kg SKF-38393 (Barnes et al., 2012). Zhu and colleagues reported an impairment in impulsivity following administration of the D1 receptor agonist SKF-38393 under standard task conditions in the 5-CSRTT (Zhu et al., 2017). The finding of the present experiment together with that of Zhu and colleagues suggests that the effectiveness of D1 receptor agonists in improving impulsivity is dependent on baseline activity of the dopaminergic system. In other words, D1 receptor activation impairs impulsivity when the levels of premature responding are low (i.e., under normal task conditions) but improves impulsivity when the levels of premature responding are high, further supporting the inverted U-shaped function of dopaminergic system manipulation on performance. D1 receptors are present at high levels in the cortex and striatum. Since waiting impulsivity in the 5-CSRT task has been mostly attributed to the mesolimbic dopaminergic pathway (Dalley and Robbins, 2017), we can predict that the improvement in waiting impulsivity following SKF-38393 administration is probably due to activation of D1 receptors in the limbic system, particularly in NAc. However, our finding contrasts with other studies that report improvement in impulsivity following administration of D1 receptor antagonist (SCH 23390) in the 5-CSRTT (Van Gaalen et al., 2006). It should be noted that the improvement in impulsivity reported by Van Gaalen and colleagues was accompanied by a reduction in the total number of trials completed, therefore, impulsivity improvement could have been secondary to a general decrease in responsivity (Gaalen et al., 2006: Shoaib and Bizarro, 2005). Indeed, other studies have reported that D1 receptor antagonists do not affect waiting impulsivity (Amalric et al., 1993; Zhu et al., 2017). Oldham's

correlation revealed that only the highest dose of SKF-38393 improved impulsivity in a baseline-dependent manner. Analysis of change in premature responding across the session revealed a gradual decrease in the number of premature responses, which represents a gradual improvement in waiting impulsivity over time. A within-session learning effect has previously been reported in studies using an extended ITI challenge with a constant SD (Young et al., 2011). In this study, all doses of SKF-38393 reduced impulsivity at the beginning of the session, however, SKF 38393 failed to improve impulsivity at the end of the session, suggesting that the improvement in performance observed following administration of SKF 38393 is due to enhanced inhibitory response control rather than faster learning of the new challenge. SKF-38393 had no effect on other performance measures such as *d* prime (sustained attention) and accuracy. SKF-38393 only showed improvement in attentional performance in the form of increased hit rate at the intermediate dose (4 mg/kg). However, the highest dose of SKF-38393 did not affect hit rate or false alarm rate. This contrasts with a previous study (Barnes et al., 2012) in which SKF 38393 produced an improvement in sustained attention at 4 and 6 mg/kg in adult rats. However, this difference could be due to the shorter range of ITI used in previous studies compared to the present study (8, 9, 10, 11 and 12 s versus 12,14 and 16 s, respectively) (Barnes et al., 2012). Therefore, the challenging conditions under which the study is conducted can be a determining feature of whether the drug improves or impairs performance.

It has been reported that patients with ADHD also suffer from lack of motivation, where their response to reward is decreased. For example, fMRI studies reported decreased responsiveness of ventral striatum (composed of nucleus accumbens core and shell) in ADHD during immediate and delayed reinforcement compared with controls (Plichta et al., 2009). Subsequent studies suggested that deficits in motivation could be due to the lower level of dopamine transporter and dopamine receptors in the nucleus accumbens of these patients compared with normal individuals (Volkow et al., 2009). Other studies further supported the association between motivation and attention by reporting that dysfunction in the mesolimbic pathway (reward), which projects from ventral tegmental area to the nucleus accumbens, is associated with lack of motivation in patients with ADHD and this deficit may contribute to their lack of attention. This finding may explain why children with ADHD are unable to pay attention to tasks they consider boring (Volkow et al., 2011; Albrecht et al., 2015). In addition, drugs commonly used to treat ADHD, for example, methylphenidate, decrease both impulsivity and excessive temporal discounting (preference for immediate reward over delayed reward) in nonhuman primates (Rajala et al., 2015). In the present study, SKF 38393 increased responsivity index at 2 and 4 mg/kg, suggesting that rats were more likely to respond to target and non-target trials. However, the highest dose of SKF 38393 did not affect responsivity index and compulsive behaviour.

Amphetamine promotes release and inhibits reuptake of catecholamine. It has been reported that amphetamine improves attentional processes and inhibitory response control in a baseline-dependent manner. For example, systemic administration of amphetamine (0.3 mg/kg) improved vigilance when baseline vigilance was low in mouse touchscreen 5C-CPT (MacQueen et al., 2018). In addition, amphetamine improved vigilance of mice exhibiting low baseline response (about 0.7 d prime) without having a significant effect on mice exhibiting high baseline response (about 1.3 d prime). In the same study, amphetamine improved impulsivity of high-impulsive mice but had little effect on impulsivity of the low-impulsive mice (Caballero-Puntiverio et al., 2019). Our findings show that administration of amphetamine, an indirect dopaminergic agonist, under high task demand (prolonged ITI) improved waiting impulsivity in a rate-dependent manner without affecting attention and response inhibition. In addition, Oldham's method revealed a limited high range effect of baseline impulsivity; high baseline values decreased with no change in the low baseline values. This finding is consistent with previous studies in the 5-CSRTT, for example, administration of amphetamine (0.3-0.9) mg/kg improved waiting impulsivity of rats (Bizarro et al., 2004; Bizarro and Stolerman, 2003). However, other studies reported that administration of amphetamine (0.3-1 mg/kg) did not

affect waiting impulsivity of mice tested under prolonged ITI with a mean of 5 s (Yan et al., 2011). Re-analysis of the present data using Oldham's method demonstrated the presence of a significant moderate inverse correlation between baseline impulsivity and the change following 1 mg/kg amphetamine (Oldham's r =0.5622*). Distribution of the data was consistent with a full range effect. Low baseline values increased while high values decreased following amphetamine administration, suggesting that the increase in low baseline values concealed the decrease in high baseline values when data was presented in the traditional way (i.e., as one group) (Bickel et al., 2016). Consistent with Yan et al. (2011), amphetamine (0.25 and 0.5 mg/kg) did not affect premature responding of mice tested under fixed inter-trial interval (5-sec) in the 5-CSRTT (Fletcher et al., 2013). Reanalysis of the data using Oldham's method revealed a low range effect consistent with rate dependence. Low baseline values increased with no change in the high baseline values (Bickel et al., 2016). The rate-dependent effect of amphetamine on inhibitory response control was consistent with effects of 6 mg/kg SKF-38393, suggesting that amphetamine modulates inhibitory response control partly through noradrenaline transporters and D1 receptors, most likely within the NAc. This finding is consistent with previous studies reporting that D1 receptors play an important role in the cognitive enhancing effects of stimulants (Arnsten and Dudley, 2005).

In summary, these findings suggest that systemic administration of the D1 receptor agonist SKF-38393 and AMPH improve waiting impulsivity in a rate-dependent manner. This study has shown an inverse relationship between baseline impulsivity and magnitude of change following administration of D1 receptor agonist or AMPH, further supporting the potential value of Oldham method in revealing rate-dependent effects of drugs in the 5C-CPT. This study is the first demonstration of enhanced inhibitory response control after selective activation of D1 receptors, suggesting that D1-selective agonists could be therapeutically beneficial in ADHD patients. In addition, effects of AMPH on waiting impulsivity further support the predictive validity of the 5C-CPT. Rate dependency may also apply to effects of SKF-38393 on other performance measures such as d prime. Further studies are required to explore the underlying neural mechanism of the rate dependent effects of SKF 38393 on waiting impulsivity.

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