Published in final edited form as:

Neuropharmacology. 2012 March ; 62(3): 1432–1441. doi:10.1016/j.neuropharm.2011.04.024.

Rats tested after a washout period from sub-chronic PCP administration exhibited impaired performance in the 5-choice continuous performance test (5C-CPT) when the attentional load was increased

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Abstract

It is well documented that schizophrenia patients exhibit dysfunction in various cognitive domains, including attention/vigilance, as demonstrated by impaired performance in the myriad of continuous performance tests (CPTs). NMDA receptor antagonists provide a pharmacological model in animals of the cognitive disruption presented in the disorder. We therefore examined the effects of a sub-chronic PCP treatment regimen (5.0 mg/kg 7-days bi-daily) in the recently developed rodent test of vigilance, the 5 Choice-Continuous Performance Test (5C-CPT). We assessed the effects of this regimen after at least a 7-day washout period on both baseline performance and when the attentional load was increased. Sub-chronic PCP-treatment impaired 5C-CPT performance in a manner consistent with impaired vigilance in patients with schizophrenia, with reduced hit rate and impaired signal sensitivity. These effects were only evident when performance was challenged following parameter manipulations. These data demonstrate that attention/vigilance is sensitive to disruption following sub-chronic PCP treatment in a pre-clinical task that may demonstrate increased analogy to human vigilance tasks. Although the PCP-induced attentional deficits are not as large as those deficits observed in other domains, these data provide evidence that this pharmacological model can affect multiple cognitive domains and may be useful for assessing putative pro-cognitive therapeutics for schizophrenia.

Introduction

Schizophrenia affects approximately 1% of the general population and is characterised by a myriad of symptoms including positive and negative symptoms as well as cognitive impairment across a number of domains (Marder et al. 2004; Paine and Carlezon Jr, 2009; Young et al. 2009a; Ibrahim and Tamminga, 2010). One domain affected and putatively core to cognitive disruption in schizophrenia is attention, specifically vigilance (Cornblatt and Keilp, 1994; Neuchterlein et al. 2008). Vigilance in humans reflects the ability to remain alert towards incoming stimulus information (Collings, 2003; Egeland et al. 2009) and is

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commonly measured using one of the many variants of the Continuous Performance Test (CPT) (Riccio et al. 2002). The CPT-Identical Pairs (CPT-IP) was chosen by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS.ucla.edu) initiative to be a part of their test battery (Neuchterlein et al. 2004; Buchanan et al. 2005) for assessing putative therapeutics for the treatment of cognitive disruption in schizophrenia. Patients with schizophrenia perform poorly in all CPT tasks, compared to healthy volunteers (Neuchterlein et al. 2008; Kern et al. 2008), including the CPT-IP (Cornblatt and Malhotra, 2001).

Attention in rodents is most commonly measured using the 5-Choice Serial Reaction Time Task (5-CSRTT) (Carli et al. 1983; Bari et al. 2008), based on Leonard's human five-choice serial reaction task (Leonard 1959; Wilkinson 1963). Various manipulations to basic 5-CSRTT parameters have been extensively utilised to challenge standard task performance, providing a valid means of further taxing attentional processing (Carli et al. 1983; Mirza and Stolerman, 1998; Stolerman et al. 2000; Grottick and Higgins 2002; Hahn et al. 2002; Hahn et al. 2003; Winstanley et al. 2003; Chudasama et al. 2003; Young et al. 2007), manipulations that include reducing the stimulus duration or extending the session length. While the 5-CSRTT has some commonalities with human CPTs (Bari et al. 2008), it consists of only target trials to which the animal must respond. The 5-CSRTT therefore lacks non-target trials requiring the inhibition of response that are present in all forms of human CPTs, limiting the application of the 5-CSRTT to accurately assess vigilance in a manner consistent with human CPTs (Robbins, 1998). Responses during non-target trials in go/no-go tasks (e.g. CPTs) may be the result of inattentiveness, stimulus discrimination inability, or poor impulse control (Eagle et al. 2008) and are important to consider, when assessing vigilance (Mackworth et al. 1952; Mackworth, 1968).

A lever-based operant task developed by McGaughy and Sarter (1995) may accurately assess vigilance, requiring animals to determine whether a visual cue appears or not prior to the presentation of the two levers. Presentation of the signal required a response on one lever, generating a hit, whilst the operation of the remaining lever represented a miss. Alternatively, if the signal was absent, pressing those same levers resulted in either a false alarm or correct rejection, respectively. As a result, the task encompasses response measures that are present in all variants of human CPTs (i.e. hits, misses, correct rejections and false alarms). However, false alarms typically constitute a failure to withhold from responding, in contrast to a correct rejection, that usually requires an inhibition of response (Eagle et al. 2008). Therefore, it could be maintained that the non-signal trial within this paradigm has limited analogy to the non-target trials presented in human CPTs, as the correct rejection requires an active response (Young et al. 2009b).

Consequently, the 5-CSRTT has recently been modified to include non-target trials, improving the similarity to human CPTs in a task known as the 5-Choice Continuous Performance Test (5C-CPT), originally validated for mice (Young et al. 2009b). As in the 5-CSRTT, target trials are presented requiring a response generating hits and misses. Importantly, the 5C-CPT also presents non-target trials, requiring the animal to correctly inhibit a response. A failure in action restraint (responding to the non-target stimulus) constitutes a false alarm. As a result, this adaptation improves the capability to assess

vigilance in a preclinical paradigm in a manner more consistent with human CPTs (Riccio et al. 2002). Although it is impossible to completely replicate the human CPT in a preclinical setting, various response outcomes are possible in the 5C-CPT (e.g. hits, misses, correct rejections and false alarms) mirroring those generated in human CPT testing. These responses enable signal detection theory (SDT) to be employed. SDT is often used in human CPT analysis (Nestor et al. 1990) and further assesses performance and provides insight into the animals' responsivity, while also generating additional information on attentional performance in light of task or pharmacological manipulations (Dudchenko et al. 1992; Marston, 1996; Steckler, 2001).

Phencyclidine (PCP) and its congener, ketamine, have been used to model aspects of schizophrenia since Luby et al. (1959) described PCP as a schizophrenomimetic drug based on observations that PCP intoxication resembled the psychotic symptoms of schizophrenia. Furthermore, it became evident that this class of drug not only induced positive-like symptoms, but also possesses the ability to mimic the negative symptomatology and are associated with cognitive disruption (both transient and persistent) in humans (Allen and Young, 1978; Carlin et al. 1978; Cosgrove and Newell, 1991; Javitt and Zukin, 1991; Krystal et al. 1994; Jentsch and Roth, 1999; Morgan et al. 2004; Morgan et al. 2010). PCP is a non-competitive NMDA receptor antagonist and its actions contributed to the development of the glutamatergic hypofunction hypothesis of schizophrenia. This hypothesis implicates dysfunctional NMDA receptor(s) and/or abnormal downstream NMDA receptor effects being core to the pathophysiology of the disease (Olney and Faber, 1995; Olney et al. 1999; Goff and Coyle, 2001), potentially providing a more compelling model than the hyperdopaminergic hypothesis of schizophrenia (Coyle, 2006).

PCP administration (both acute and sub-chronic) disrupts cognitive performance in animals in a variety of paradigms (see Neill et al. 2010 for review), providing support that NMDA receptor antagonism may model cognitive impairment exhibited in schizophrenia (Jentsch et al. 1999). In animals, in several paradigms assessing the various domains of cognition affected in schizophrenia, a sub-chronic PCP treatment regimen (twice daily for 7 days followed by at least 7 days drug-free) has been shown to induce robust and long-lasting cognitive impairment (Neill et al. 2010).

NMDA receptor antagonism can disrupt attentional processing, demonstrated when animals were acutely or repeatedly administered PCP, and tested while under the influence of PCP (Amitai et al. 2007; Amitai and Markou, 2009). Additionally, intra-medial prefrontal cortical (mPFC) infusion of the competitive NMDA antagonist, 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP) also disrupts 5-CSRTT performance (Murphy et al. 2005; Baviera et al. 2008). These data further highlight the involvement of the NMDA receptor in attentional function and moreover, implicate the mPFC in attentional processing.

To date, studies have only shown disruption of 5-CSRTT performance while animals were 'acutely' intoxicated with NMDA antagonists (Le Pen et al. 2003; Amitai et al. 2007; Amitai and Markou, 2009; Auclair et al. 2009) and not following a significant washout period. In addition to cognitive impairment, sub-chronic PCP administration followed by a significant washout period has been shown to induce a number of neurochemical, morphological and

metabolic disturbances in the drug free state, mirroring alterations evident in pathology of the disorder (Jentsch et al. 1997a; Jentsch et al. 1997b; Cochran et al. 2003; Hajszan et al. 2006; Abdul-Monim et al. 2007; Jenkins et al. 2008; Snigdha et al. 2010). These findings are not evident following acute administration (Jentsch and Roth, 1999; Cochran et al. 2003). Due to the paucity of information regarding attentional impairment in the drug-free state following a sub-chronic PCP treatment regimen and 5-CSRTT performance, there is clearly a need to understand the enduring effects of long-term NMDA antagonism on attention/vigilance. Thus, the purpose of the present study was to determine the effect of sub-chronic PCP treatment followed by at least a 7-day washout period, on performance in the 5C-CPT, at both baseline conditions and when the attentional load of the task was increased.

2. Methods and Materials

2.1 Subjects

Female hooded-Lister rats (n=35; Charles River; approx 220 ± 10 g at the start of the experiment) were housed in groups of five on a 12 hour reversed light cycle (lights on at 7:00pm) in a temperature ($21 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$) controlled environment. All experimentation took place in the dark cycle, under red lighting, and animals had free access to food (Special Diet Services, UK) and water until one week prior to the beginning of training, where food restriction reduced animal weight to 90% of the free-feeding body weight. Food restriction continued throughout training and testing, but water was available *ad libitum* whilst in the home cage. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) 1986 Act and local University of Bradford ethical guidelines.

2.2 Apparatus

The test apparatus consisted of eight $25\text{ cm} \times 25\text{ cm}$ aluminium chambers, each enclosed within a wooden sound attenuating box (constructed in-house at GlaxoSmithKline, GSK Harlow, UK). The rear wall of the testing chamber was concavely curved and contained nine individual apertures, four of which were occluded, leaving apertures 1, 3, 5, 7 and 9 free for exploration. Each aperture was 2.5 cm^2 , 4 cm deep and set 2 cm above floor level. Located at the rear of each aperture was a white LED, which provided the light stimulus. At the entrance to each aperture, an infrared photocell beam registered nose-poke responses. Located on the front wall of the test chamber was a food magazine that allowed retrieval of the food reward (45 mg Rodent Pellet, Sandown Scientific). A hinged panel covered the food magazine and a micro-switch reported the collection of food rewards. In addition to the sound attenuating box, there was also a low-level fan that provided ventilation along with a means to mask extraneous background noises. The floor of the test chamber consisted of a wire grid, under which was a removable tray that was covered with sawdust. All eight chambers were connected to a PC and data collection and initial analysis was controlled by K-Limbic software (Conclusive Solutions).

2.3 Behavioural Procedure

The 5C-CPT is similar to the 5-CSRTT which is described in detail elsewhere (Grannon et al. 2000; Robbins, 2002; Chudasama and Robbins, 2004). Briefly, rats were trained to detect

and report the occurrence of a brief visual stimulus (1 s; Mirza and Stoleran, 1998; Amitai et al. 2007; Amitai and Markou, 2009; Fletcher et al. 2007; Young et al. 2007) presented pseudo-randomly (maximum of 2 consecutive presentations within a single aperture) in one of the five available apertures following a variable (4.0, 4.5, 5.5, and 6.0 s) inter-trial interval (ITI), conducted with the house light extinguished. Correct detection of the visual stimulus within the limited hold (LH; time period following the stimulus during which animals can make a response, 2 s) was reported via a nose-poke in the aperture the stimulus was presented in (**correct response**), resulted in a food reward. Reward collection immediately initiated the next ITI. Failure to respond to the stimulus (**error of omission**) or responding to an aperture where the light was not presented (**incorrect response**) resulted in the animal being exposed to a 5 s time out (TO) period, whereby the house light was illuminated and no food reward delivered. Failure to wait for the stimulus presentation, i.e. a nose-poke during the ITI, was recorded as a **premature response** and was also followed by a TO period. Animals also received a TO period following a repeat response in any of the apertures (**perseverative response**). Subsequent responses made during the TO restarted the 5 s TO period and were recorded as **time out responses**. The 5C-CPT procedure also included non-target trial stimuli that appeared as lights presented in all apertures, whereby the animal must inhibit from responding (**correct rejection**) in order to obtain a reward. Responses made within the LH period, during a non-target trial (**false alarm**), resulted in a TO period (Young et al. 2009b). The session consisted of 120 trials, lasting no more than 30 minutes. Several calculations that are available in the 5-CSRTT (accuracy, omissions, latencies etc; see table 1) are available in the 5C-CPT, while measures commonly included in human CPTs (hit rate false alarm rate, sensitivity index, responsivity index; see table 1) are also assessed to characterise animal performance. In previous 5-CSRTT studies, accuracy is also referred to as percent correct responding and the terms have been used interchangeably. These two measures are distinguishable, however, because accuracy is calculated independently of omissions, whereas percent of correct responding is calculated from the total number of responses within the session (correct, incorrect and omissions). We have adopted this method of calculating accuracy and percent correct responses, as previously described by others (Amitai et al. 2007; Amitai and Markou, 2009).

The following describes the method used to calculate the indices used in signal detection analysis as originally described by Frey and Colliver (1973), also used in the mouse 5C-CPT (Young et al. 2009b). Correct, incorrect and omissions refer to possible response outcomes when presented with target trial, FA indicates an incorrect response to non-target trial and CR refers to a correct rejection of non-target trial:

$$p[HR] = \frac{Correct}{Correct + Incorrect + Omission} \quad p[FA] = \frac{FA}{FA + CR}$$

$$SI = \frac{p[HR] - p[FA]}{2(p[HR] + p[FA]) - (p[HR] + p[FA])^2}$$

$$RI = \frac{p[HR] + p[FA] - 1}{1 - (p[FA] - p[HR])^2}$$

2.4 Training Schedule

Training was conducted in a similar manner to the original 5-CSRTT (Bari et al. 2008), with the exception that the modified task included non-target trials, whereby all apertures presenting a visual stimulus and the reward pellet was dispensed following a correct rejection constituting an inhibition of response. At first, there was greater emphasis placed on the presentation of non-target trials as the proportion per session was increased (77 target: 43 non-target) until rats could correctly respond to target trials and inhibit a response to non-target trials. Once rats were able to reliably discriminate between trial types ($p[HR] > p[FA]$), the proportion of target trials per session was increased to 84 and non-target trials reduced to 36, a configuration used throughout the remainder of training and testing. At the start of training the SD was 10 s and was progressively reduced (10, 8, 4, and 2 s) for individual rats as they reached criterion ($>75\%$ accuracy, $<25\%$ omission and $>65\%$ correct rejections of non-target trials, for three consecutive days). Training was conducted until animals achieved a stable performance at the desired testing parameters (1 s SD, 5 s TO, variable ITI with a mean of 5 s and 2 s LH) over three consecutive days. It took approximately 3.5 months for the animals to reliably discriminate between trial types and a further 1.5 – 2.5 months for animals to achieve stable performance at the desired criteria, totalling approximately 5 – 6 months for the entire training procedure.

2.5 Drug Treatment Regimen

Rats were divided into three treatment groups, matched for baseline performance. Following group-matching, rats were sub-chronically administered PCP (2.5 mg/kg, $n = 11$ or 5.0 mg/kg, $n = 10$ i.p.) or vehicle (0.9% saline, $n = 11$) twice daily (9am and 5pm) for 7-days, followed by at least a 7-day washout period, after which behavioural testing commenced. Animals were not trained or tested during drug treatment. Rats that failed to reach a stable baseline at the desired criteria were excluded from the study ($n = 3$).

2.6 Experimental Design

2.6.1 Stable Baseline Performance—Rats were tested following sub-chronic PCP administration (8-days post PCP) using standard training parameters that consisted of a 1 s SD, variable ITI which had a mean of 5 s (4.0, 4.5, 5.5, and 6.0 s), a 5 s TO period and a 2 s LH. The animals were presented with 84 target trials and 36 non-target trials in a session lasting no more than 30 minutes.

2.6.2 Variable Stimulus Duration—Rats performance was challenged (9-days post PCP regimen) within a single session that presented a variable SD (0.25, 0.5, 0.75, and 1 s) as opposed to a fixed SD (1 s). All other parameters (TO, LH go/no-go ratio) were as before with the exception of the variable ITI, which due to programming limitations, was switched to a fixed ITI (5 s).

2.6.3 Extended Session—Rats were exposed to an extended session (11-days post PCP regimen) to further challenge vigilance performance. The session consisted of 256 trials, of which 160 were target (40×4 ITIs) and 96 non-target trials (24×4 ITIs) and lasted no longer than 60 minutes. Test parameters were the same as standard training conditions i.e. 1 s SD, variable ITI (4.0, 4.5, 5.5, and 6.0 s), 5 s TO and 2 s LH.

All animals received a normal training session between the vSD and extended session protocol manipulations to ensure performance was maintained at a stable baseline.

2.7 Data Analysis

All data were displayed as observed mean $\pm$ SEM. Overall performance was analysed by one-way between-subjects ANOVA followed by Planned Comparisons. Analysis of the performance across the duration of the session was carried out by two-way repeated measures ANOVA (Treatment as a fixed factor and Trial as a repeated measures factor – session was divided into trial bins, each composed of 40 trials for the 120 trial sessions or 64 trials for extended session) followed by Planned Comparisons (Snedecor and Cochran, 1989). Assessment of the effect of a variable SD on task performance was conducted by two-way repeated (SD as within-subjects factor and Treatment as between-subjects factor) measures ANOVA, followed by Planned Comparisons. Assessment of vSD on performance of control animals' was conducted by one-way repeated measures ANOVA (SD as within-subjects factor). Alpha level was set to 0.05 and all analysis was carried out using Statsoft Statistica (v8.0).

Results

3.1 Stable Baseline Performance

A one-way ANOVA analysis revealed sub-chronic PCP treatment had no significant effect on baseline performance for any measure (table 2).

A two-way repeated measures analysis of the baselines also revealed the performance did not alter with session (data not shown). No significant Dose*Trial interaction were observed for any measure.

3.2 Variable Stimulus Duration (vSD)

3.2.1 Overall Performance—A vSD was then used to challenge performance (table 3). Statistical analysis revealed no significant difference in accuracy between treatments [$F_{(2,29)}=2.01$, NS] though Planned Comparisons analysis demonstrated there was a trend towards reduced accuracy in the 5 mg/kg PCP treatment group ($p=0.06$) when compared to the vehicle group. A significant effect of treatment on percent correct responding [$F_{(2,29)}=5.85$, $p<0.01$] was observed with PCP (5 mg/kg) significantly reducing percent correct responses ($p<0.01$). A strong trend toward a significant PCP effect was observed for percent omissions [$F_{(2,29)}=3.16$, $p=0.057$], with Planned Comparisons showing that PCP (5 mg/kg) significantly increased percent omissions ($p<0.05$).

There was no significant overall PCP treatment effect on latency to respond (CL, IL, or ML) or perseverative responses although increased TO responses [$F_{(2,29)}=4.12$, $p<0.05$] were

observed (5 mg/kg) when compared with vehicle ($p < 0.01$). There was no significant effect on the number of premature responses [$F_{(2, 29)} = 2.36$, NS], although there was a trend towards a significant effect at the highest dose ($p = 0.07$).

PCP treatment significantly reduced $p[HR]$ [$F_{(2, 29)} = 5.85$, $p < 0.01$] with Planned Comparisons revealing that 5 mg/kg PCP significantly reduced $p[HR]$ when compared with vehicle treatment ($p < 0.01$). There was a trend towards an increase in the $p[FA]$ [$F_{(2, 29)} = 2.78$, $p = 0.07$] with the highest dose of PCP significantly increasing $p[FA]$ ($p < 0.05$). Sub-chronic PCP treatment significantly decreased the SI [$F_{(2, 29)} = 6.83$, $p < 0.01$] with both 2.5 mg/kg and 5 mg/kg PCP significantly reducing signal discrimination ($p < 0.05$ and $p < 0.001$ respectively), while there was no significant effect on the RI [$F < 1$, NS].

3.2.2 Within-Session Performance—Within-session analysis of performance from the vSD challenge revealed some more specific effects of sub-chronic PCP compared to overall performance (fig 1). A repeated measures ANOVA approach revealed a Dose*Trial interaction on accuracy [$F_{(4, 58)} = 3.45$, $p < 0.05$]. Planned Comparisons revealed that 2.5 mg/kg PCP reduced accuracy in the first part of the session ($p < 0.05$), whilst 5 mg/kg PCP reduced accuracy in the final part of the session ($p < 0.05$), when compared to vehicle treated animals. PCP treatment resulted in no Trial effect [$F_{(2, 58)} = 2.21$, NS] and no Trial*Treatment interaction [$F_{(4, 58)} = 0.28$, NS] on percent omission. However, Planned Comparisons demonstrated 5 mg/kg significantly increased omissions, compared to control animals, in the first trial bin ($p < 0.05$).

PCP treatment reduced $p[HR]$ [Treatment: $F_{(2, 29)} = 5.71$, $p < 0.01$, Treatment*Trial: $F_{4, 58} = 1.75$, NS]. Planned Comparisons demonstrated that 2.5 mg/kg and 5 mg/kg significantly reduced the $p[HR]$ during the first trial bin ($p < 0.05$). The highest dose of PCP (5 mg/kg) produced a trend towards a significant reduction in $p[HR]$ in the medial group of trials ($p = 0.058$) but resulted in a significant reduction in $p[HR]$ in the latter trial bin ($p < 0.05$). PCP treatment resulted in a trend toward increased $p[FA]$ [Dose: $F_{(2, 29)} = 2.84$, $p = 0.07$, Dose*Trial: $F_{4, 58} = 1.26$, NS]. Planned Comparisons showed that PCP (2.5 & 5 mg/kg) significantly elevated $p[FA]$ in the last group of trials in the session ($p < 0.05$ and $p < 0.01$, respectively). In light of the effect on $p[HR]$ and $p[FA]$, it was clear that PCP produced a significant Trial*Dose interaction for SI [$F_{(4, 58)} = 2.80$, $p < 0.05$], with Planned Comparisons indicating that 5 mg/kg PCP significantly reduced SI in the first two trial bins ($p < 0.05$), with a more substantial reduction observed in the last group of trials when compared with vehicle treatment ($p < 0.001$). PCP treatment had no significant effect on the RI [$F_{4, 58} = 1.38$, NS].

While there was no significant difference between treatment groups in RI for each trial bank, a significant reduction of RI in vehicle treated rats was observed as the session progressed [$F_{(2, 29)} = 4.08$, $p < 0.05$]. A significant reduction between the RI of trials 0 – 40 when compared to trials 81 – 120 ($p < 0.01$), which was not present in PCP treated animals (Table 4).

3.2.3 Effect of vSD on performance—There was a significant main effect of SD on response accuracy [$F_{(3, 87)} = 56.39$, $p < 0.001$] and percent omissions [$F_{(3, 87)} = 29.23$, $p < 0.001$]

when a vSD was introduced, without a significant interaction between SD and Dose for accuracy [$F_{(6,87)}=0.3$, NS] or percent omissions [$F_{(6,87)}=0.4$, NS]. There was a main effect of SD on p[HR] [$F_{(3,87)}=84.44$, $p<0.001$], but no significant interaction between SD and Treatment [$F_{(6,87)}=0.2$, NS]. No significant effect of SD was observed on p[FA] [$F_{(3,87)}=0.8$, NS] although there was a slight trend towards a SD*Treatment interaction [$F_{(6,87)}=1.86$, $p=0.09$] (data not shown). There was a significant main effect of SD on the sensitivity index [$F_{(3,87)}=23.32$, $p<0.001$], along with a significant SD*Treatment interaction [$F_{(6,87)}=2.48$, $p<0.05$], with Planned Comparisons demonstrating a significant PCP-induced reduction in SI following 1 s SD (5 mg/kg, $p<0.05$), 0.75 s SD (2.5 and 5 mg/kg, $p<0.05$) and 0.25 s SD (2.5mg/kg, $p<0.05$; 5 mg/kg, $p<0.001$). Additionally, when the effect of SD on SI was analysed specifically for control animals, a main effect was observed [$F_{(3,30)}=4.95$, $p<0.01$], with Planned Comparisons showing that SI following 0.25 s SD was significantly reduced compared with SI following 1 s SD ($p<0.05$) (fig 2). There was significant main effect on the responsivity for SD [$F_{(3,87)}=26.38$, $p<0.0001$], but no interaction of SD*Dose [$F_{(6,87)}=0.9$, ns] (data not shown).

3.3 Normal Baseline Performance

Following the vSD challenge, but prior to the extended session challenge (day 10), performance was assessed when protocols were returned to that of baseline. Animal performance returned to baseline with no significant differences existing between treatment groups in any of the behavioural measures assessed (data not shown).

3.4 Extended Session

3.4.1 Overall Performance—The extended session challenge (table 5), revealed varying effects of PCP treatment. There was no overall effect on accuracy [$F_{(2,29)}=1.38$, NS], percent correct [$F_{(2,29)}=1.41$, NS] or percent omissions [$F_{(2,29)}=1.05$, NS]. A trend toward significance was observed for CL [$F_{(2,29)}=3.17$, $p=0.056$], with Planned Comparisons indicating that 2.5 mg/kg PCP reduced CL ($p<0.05$). No significant effect was observed for ML [$F_{(2,29)}=1.93$, NS] or IL [$F_{(2,29)}=1.2$, NS]

There was a trend towards a significant increase in perseverative responding [$F_{(2,29)}=2.68$, $p=0.08$], with Planned Comparisons revealing that 5 mg/kg PCP significantly increased perseveration ($p<0.05$). Additionally, there was also a significant increase in TO responses [$F_{(2,29)}=3.74$, $p<0.05$] and Planned Comparisons indicated that 5 mg/kg PCP increased TO responding ($p<0.05$). No effect on premature responses were observed [$F_{(2,29)}=2.40$, NS].

Sub-chronic PCP produced no significant effect on p[HR] [$F_{(2,29)}=1.41$, NS], but increased p[FA] [$F_{(2,29)}=3.57$, $p<0.05$], by both doses of PCP following Planned Comparisons analysis ($p<0.05$). There was an overall reduction in the SI [$F_{(2,29)}=6.84$, $p<0.01$] with Planned Comparisons indicating both 2.5 and 5 mg/kg decreasing discriminability ($p<0.05$ and $p<0.01$, respectively). No effect of PCP was observed for RI [$F_{(2,29)}=1.00$, NS].

3.4.2 Within-Session Performance—In a within-session analysis of the extended session challenge (fig 3) on performance repeated measures ANOVA revealed that sub-chronic PCP treatment did not affect accuracy [$F_{(6, 87)}=1.1$, NS]. A Trial effect was observed

for percent omissions [$F_{(3,87)}=13.03$, $p<0.001$], attributed to an increase in omissions in trial bin 4 ($p<0.001$) (data not shown). Additionally, following the extended session (fig 3), Planned Comparisons revealed 2.5 mg/kg induced a significant reduction in CL in the first three trial groups ($p<0.05$) and a trend towards a significant reduction in the final group of trials ($p=0.06$). PCP treatment produced no significant overall effect on ML [$F_{(6,87)}=0.39$, NS]. A Trial effect was observed for p[HR] [$F_{(3,87)}=14.51$, $p<0.001$]. A Treatment*Trial interaction was observed for p[FA] [$F_{(6,87)}=2.90$, $p<0.05$]. The significant increase in p[FA] was associated with 2.5 and 5 mg/kg PCP during the first group of trials ($p<0.01$ and $p<0.05$, respectively) and 5 mg/kg PCP in the second group of trials ($p<0.01$). PCP did not increase p[FA] in remaining trial groups. Although there was no Treatment*Trial interaction [$F_{(6,87)}=1.47$, NS], there was an overall Trial effect on SI [$F_{(3,87)}=8.73$, $p<0.001$]. ANOVA initially demonstrated Drug did not significantly affect RI [$F_{(2,29)}=2.40$, NS]. There was a Trial [$F_{(3,87)}=10.41$, $p<0.001$] and a Trial*Treatment interaction on RI [$F_{(6,87)}=2.57$, $p<0.05$] and Planned Comparisons revealed 2.5 mg/kg and 5 mg/kg PCP increased RI in the first trial bin ($p<0.01$ and $p<0.05$, respectively).

3.4.3 Vigilance Decrement—Analysis of the control animals' performance demonstrated a trend towards a main effect when SI was analysed by repeated measures (trial as within-subjects factor) ANOVA [$F_{(3,30)}=2.58$, $p=0.07$], with Planned Comparisons indicating that Trial bin 4 was significantly reduced ($p<0.05$) when compared with Trial bin 2 (fig 4).

Discussion

The present study supports the hypothesis that attentional impairment is evident following a sub-chronic PCP treatment regimen and at least a 7-day washout period. The performance disruption observed is consistent with impaired vigilance seen in schizophrenia patients and was evident up to 11 days after cessation of treatment. Importantly, here we use the 5C-CPT, which may have improved translational validity for assessing vigilance in rodents consistent with CPT testing in humans (Young et al. 2009b). These data also indicate that elucidation of these deficits requires challenging performance beyond that of the baseline task. Hence, in some cases the attentional impairment only became evident when the performance was displayed in discrete time bins throughout the session. To our knowledge, this is the first time that sub-chronic PCP-induced impairments in the pre-clinical test of vigilance, the 5C-CPT, have been demonstrated in rats.

Successful performance of the 5C-CPT requires animals to be thoroughly trained with a highly pre-potent response necessary for task completion. While acute or repeated PCP treatment resulted 5-CSRTT deficits at baseline (Amitai et al. 2007; Amitai and Markou, 2009), the lack of impairment on baseline performance in the present study suggests that a sub-chronic PCP treatment regimen does not lead to task disruption when performance was assessed at baseline attentional demands following a 7-day washout period. Although this is in contrast to previous investigations, a major methodological difference exists; previous studies were conducted when animals were under the influence of PCP treatment (Le Pen et al. 2003; Amitai et al. 2007; Amitai and Markou, 2009; Auclair et al. 2009) and not in the drug-free state after a significant washout period, observed in the current investigation. Normal baseline performance in the 5-CSRTT following dorsal noradrenergic bundle lesions

(Carli et al. 1983; Cole and Robbins, 1992) or dopamine depletion of the dorsal striatum (Baunez and Robbins, 1999), have been observed previously with deficits only apparent during task challenges, which included reducing stimulus intensity, stimulus duration, event-rate, or interpolating bursts of distracting white noise (see Robbins, 2002). These findings prompted the use of behavioural challenges in the 5C-CPT in order to elucidate performance deficits induced by sub-chronic PCP treatment.

By manipulating the test parameters to further tax vigilance performance, PCP treated animals had a reduced ability to perform the 5C-CPT, compared to control animals. The variable SD (vSD) task challenge was chosen specifically because it does not fundamentally alter the protocol the animal is responding to. Thus, changes in performance are less likely to reflect within-session learning, as has been observed elsewhere (Hahn et al. 2002; see Young et al. 2009a). When a vSD was introduced, so increasing the attentional demands of the task (McGaughy and Sarter, 1995; Jones et al. 1995; Mirza and Stoleran, 1998; Parasuraman, 1998), animals treated with PCP exhibited a reduced performance consistent with attentional dysfunction. A reduction in percent correct responding was observed, coupled with an increase in trials omitted, consistent with previous reports using the standard 5-CSRTT (Amitai and Markou, 2010).

The addition of non-target stimuli in the 5C-CPT provides a means to assess this deficit. In comparison with control animals, PCP treatment also reduced the rats' ability to withhold a response when presented with non-target stimuli, suggesting impairment in action restraint (Eagle et al. 2008). Although, it appeared as if PCP treatment did not result in behavioural *disinhibition*, increasing p[FA], but impaired the development of behavioural *inhibition* when presented with non-target trials. Therefore, PCP-treatment is not increasing inappropriate responding, but rather impairing the reduction of inappropriate responding, an effect which occurs in control animals. This effect, coupled with the PCP-induced reduction in p[HR] in the drug-free state, ultimately reduced the sensitivity index (SI). The SI has been implemented as a non-parametric method of displaying d' commonly used in human CPTs to assess performance, reflecting a measure of vigilance. D' is consistently reduced in schizophrenia patients indicating that a discrimination decrement is inherent to the disorder (Nestor et al. 1990; Parasuraman, 1998; Mass et al. 2000; Baerwald et al. 2005; Wang et al. 2007; Park et al. 2010). The PCP-induced reduction of SI, following the vSD challenge, is consistent of the impaired performance of patients with schizophrenia in human CPTs. Additionally, healthy volunteers infused with ketamine demonstrated impairment in CPT performance, exemplified by a decreased hit rate, increased false alarm rate and impaired signal sensitivity (Krystal et al. 1994; Umbricht et al. 2000; Heekeren et al. 2008). However, these studies assessed acute ketamine exposure and did not investigate the enduring effect of chronic ketamine use on CPT performance.

The presence of target and non-target stimuli in the 5C-CPT also provides the ability to assess response strategy (bias). Changes in SI (as described above) are less likely to reflect deficits of the construct being measured (vigilance) if the change was due to alterations in bias (Marston, 1996). The reduction in SI in the present study was not accompanied by alterations in the responsivity index measure of bias (RI) between treatment groups. Thus it is likely that PCP treatment truly resulted in impaired vigilance observed as a reduced ability

of animals to discriminate between signal and noise, consistent with reduced d' exhibited by schizophrenia patients in human CPTs. It was apparent that the RI of control animals significantly reduced as the session progressed, suggesting that animals were modifying their strategy in light of the increased cognitive demands of the challenge, an effect that was absent in PCP treated animals. These data, therefore, suggest that PCP treatment may have reduced the ability of animals to adapt to the increased attentional load of the task, which may account for the reduced performance when challenged with a vSD. Vehicle treated rats' p[FA] progressively improved as the session progressed, possibly as a result of adaptation to the challenge of varying SDs or consistent, and therefore more predictable ITIs, which was reflective of their more conservative bias over time. Varying the SD in mice while maintaining a variable ITI does not result in improved performance over the session (J. Young unpublished observations) suggesting that, in the present studies, rats may be honing a temporal mediating strategy (Spratt et al. 2000) within the session, consistent with previous reports when using extended ITI challenges (Hahn et al. 2002). Noradrenergic depletion also impairs rats' ability to adapt to variable ITI challenges (when trained with a constant ITI) in the 5-CSRTT (Cole and Robbins, 1992), while noradrenaline levels as measured by microdialysis only increase during such challenges (Dalley et al. 2001). Thus PCP-induced deficits in the vSD challenge are possibly the result of inhibiting the adaptation of the better strategy and may reflect alterations in noradrenergic levels.

Evidence for a PCP-induced accentuation of a vigilance decrement, or cognitive fatigue was observed when performance was examined across the session. Response accuracy (a common measure of sustained attention, Robbins, 2002; Amitai et al. 2007; Bari et al. 2008; Amitai and Markou 2010) was reduced and the p[FA] was increased in PCP-treated rats compared to the control group at the end of the session. Furthermore, the reduction in SI became more substantial in the latter part of the session. These data suggest that PCP treatment impaired 5C-CPT performance, which became more prominent as the session progressed. Along with the deficit in attentional performance, PCP-treated rats also exhibited increased TO responding in PCP treated animals, which may reflect a form of cognitive inflexibility (Amitai and Markou, 2010; Dalley et al. 2002).

Vigilance was further challenged by extending the session duration. The most robust finding was a PCP-induced reduction in SI driven primarily by increased p[FA] indicative of response disinhibition. During the start of the session (trial 0 – 64) this was coupled with increased RI, indicating a more liberal strategy than vehicle treated animals and the PCP-treated animals may simply have been increasing their responding to all signals. The initial impairment in performance may have been due to a shift in decision criteria, as opposed to alterations in cognitive processing *per se*. Alterations in RI disappeared as the session progressed, while p[FA] remained elevated. Moreover the p[HR] was unaffected, suggesting that the animals were not simply hyper-responsive to all stimuli. Furthermore, the PCP-induced SI decrement was only evident in the middle part of the session (trials 65 – 192), independent of changes in RI, indicative of reduced performance resulting from cognitive dysfunction (Dudchenko, 1992; Marston, 1996). While the PCP-induced impairment in SI was absent in the latter part of the session (trial 193 – 256), this was primarily due to a reduction in SI in the vehicle treated animals. This is likely due to cognitive fatigue in control animals, demonstrated by the significant reduction in SI from trial bin 2 to trial bin 4,

producing a reduced discrimination of trial types, suggestive of a vigilance decrement consistent with many human CPTs (Parasuraman, 1998; Riccio et al. 2002) and mice in the extended session 5C-CPT challenge (Young et al. 2009b). Although impaired performance towards the latter part of the session may be due to satiety, the lack of reward latency changes across the session suggests that motivation remained consistent (Robbins, 2002) thus satiation is unlikely, further supporting the hypothesis that a vigilance decrement was observed. The vigilance decrement observed with the extended session challenge is in contrast to improved performance over time in the vSD challenge supporting the use of an extended session challenge to further probe vigilance. The extended session challenge also revealed sub-chronic PCP-induced reduction in correct latency (CL), without affecting reward or incorrect latencies. This is in contrast to PCP-induced slowing of responding observed following acute administration, although the effects following acute treatment were likely to be non-specific motoric effects as other latency measures were also slowed (Le Pen et al. 2003; Amitai et al. 2007). Interestingly, acute administration of MK-801 has been shown to reduce CL in the 5-CSRTT (Grottick and Higgins, 2000), an effect that was absent at a higher dose. Thus, NMDA antagonism (either acute or sub-chronic) may reduce CL in a dose specific manner.

A number of potential mechanisms may underpin the PCP-induced deficit that is observed following a behavioural challenge. Firstly, glutamatergic transmission within the PFC is impaired following sub-chronic PCP treatment in the drug-free state (Murai et al. 2007), with cortical glutamate transporter expression increased, reducing cortical glutamate levels (Fattorini et al. 2008). Evidence also exists for a reciprocal function between the dopaminergic and glutamatergic systems (Carlsson, 1995) and it has been demonstrated that following sub-chronic PCP treatment, dopamine (DA) utilization in the prefrontal cortex (PFC) is impaired (Jentsch et al. 1997b; Jentsch and Roth, 1999). The glutamatergic and dopaminergic dysfunction in frontal cortical regions may be central to the cognitive dysfunction following drug treatment. However, these findings cannot account for the lack of impairment under baseline. Acetylcholine (ACh) has been implicated in attentional processing and modulation (Robbins, 2002; Dalley et al. 2004; Herrero et al. 2008). Although, following sub-chronic PCP treatment it has been shown that there are no changes in basal levels of ACh in the drug free state in the PFC (Jentsch et al. 1998). The lack of impaired prefrontal ACh utilisation may account for the normal baseline performance in the task seen following PCP treatment. It is evident that the cholinergic system has interactions with the GABAergic system (Muir et al. 1994; Sarter, 1994) and it has been documented that GABAergic interneurons are sensitive to NMDA receptor antagonism, characterised by a reduction in parvalbumin containing neurones (Cochran et al. 2003; Abdul-Monim et al. 2007). A sub-chronic PCP-induced reduction in GABAergic interneuron function may reduce the capacity of the prefrontal cortex to recruit ACh when the rat is faced with an increase in cognitive demands. Thus, sub-chronic PCP-induced impairment in vigilance is only observed with increased attentional load. Alternatively, impairments in the present study are similar to those observed by Carli et al. (1983) following DNAB lesion and behavioural challenge. It is therefore possible that impairment following parameter manipulation in PCP treated animals' resulted from dysfunctional noradrenergic transmission. As previously discussed, NA has been implicated in the formation of strategy

(Dalley et al. 2001); with abnormal noradrenergic transmission potentially accounting for the impairment in strategic adaptation observed in PCP treated animals following vSD challenge. Future studies examining the correlation between vigilance and parvalbumin containing neurones, the effects of DNAB lesions or white noise distracters in PCP treated animals could elucidate this possibility, providing information regarding the potential mechanism(s) underlying impaired performance.

In summary, these data demonstrate that sub-chronic PCP treatment, followed by at least a 7-day washout period, resulted in performance deficit in the 5C-CPT. Importantly, the PCP-induced attentional impairment was only detected following an increase in the attentional load. These data therefore suggest that the cognitive domain of attention/vigilance, although susceptible, may be less sensitive to the long-term disruptive effects of sub-chronic PCP treatment regimen in the drug-free state. This conclusion is made when the PCP-induced impairments are compared to other domains of cognition (such as episodic memory, cognitive flexibility, social cognition and executive functioning), which are clearly disrupted in the drug-free state following sub-chronic PCP treatment, without the need for further task challenge or drug treatment (see Neill et al. 2010 for a full review). It may be that PCP-induced deficits in these tasks are mediated via a different mechanism to those observed in the current study, although further studies will be required to test this hypothesis.

Acknowledgments

The authors would like to thank Dr Simon Bate for his most helpful and consistent advice regarding the statistical design and analysis. Support was provided by the University of Bradford postgraduate studentship (SAB) and by NIMH grant R21-MH85221 (JWY)

References

- Abdul-Monim Z, Neill JC, Reynolds GP. Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbuminimmunoreactive expression in the rat. *J Psychopharmacol.* 2007; 21:198–205. [PubMed: 17329300]
- Allen RM, Young SJ. Phencyclidine-induced psychosis. *Am J Psychiat.* 1978; 135:1081–1084. [PubMed: 696930]
- Amitai N, Semenova S, Markou A. Cognitive disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medication in rats. *Psychopharmacology (Berl).* 2007; 193:521–537. [PubMed: 17497138]
- Amatai N, Markou A. Chronic nicotine improves cognitive performance in a test of attention but does not attenuate cognitive disruption induced by repeated phencyclidine administration. *Psychopharmacology.* 2009; 202:275–286. [PubMed: 18618099]
- Amatai N, Markou A. Disruption of performance in the five-choice serial reaction time task induced by administration of N-Methyl-D-Aspartate receptor antagonists: Relevance to cognitive dysfunction in schizophrenia. *Biol Psychiatry.* 2010; 68:5–16. [PubMed: 20488434]
- Auclair AL, Besnard J, Newman-Tancredi A, Depoortere R. The five choice serial reaction time task: Comparison between Sprague-Dawley and Long-Evans rats on acquisition of the task, and sensitivity to phencyclidine. *Pharmacol Biochem Behav.* 2009; 92:363–369. [PubMed: 19353758]
- Baerwald JP, Tyron WW, Sandford J. Bimodal response sensitivity and bias in a test of sustained attention contrasting patients with schizophrenia and bipolar disorder to normal comparison group. *Archives of Clinical Neuropsychology.* 2005; 20:17–32. [PubMed: 15620812]
- Bari A, Dalley JW, Robbins TW. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature Protocols.* 2008; 3(5): 759–767. [PubMed: 18451784]

- Baunez C, Robbins TW. Effects of dopamine depletion in the dorsal striatum and further interaction with subthalamic nucleus lesion in an attentional task in rats. *Neuroscience*. 1999; 92:1343–1356. [PubMed: 10426489]
- Baviera M, Invernizzi RW, Carli M. Haloperidol and clozapine have dissociable effects in a model of attentional performance deficits induced by blockade of NMDA receptors in the mPFC. *Psychopharmacology*. 2008; 196:269–280. [PubMed: 17940750]
- Broberg BV, Glenthøj BY, Dias R, Larsen DB, Olsen CK. Reversal of cognitive deficits by an ampakine (CX515) and sertindole in two animal models of schizophrenia – sub-chronic and early postnatal PCP treatment in attentional set-shifting. *Psychopharmacology (Berl)*. 2009; 206(4):631–640. [PubMed: 19390843]
- Buchanan RW, Davis M, Goff D, Green MF, Keefe RSE, Leon AC, Nuechterlein KH, Laughren T, Levin R, Stover E, Fenton W, Marder SR. A summary of the FDA-NIMH-MATRICES workshop on clinical trial design for neurocognitive drugs for schizophrenia. *Schizophr Bull*. 2005; 31(1):5–19. [PubMed: 15888422]
- Carli M, Robbins TW, Evenden JL, Everitt BJ. Effects of lesions to ascending noradrenergic neurones on performance on a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav Brain Res*. 1983; 9:361–380. [PubMed: 6639741]
- Carlin AS, Grant I, Adams KM, Reed R. Is phencyclidine (PCP) abuse associated with organic mental impairment? *Am J Drug Alcohol Abuse*. 1978; 6(3):273–281.
- Chudasama Y, Passetti F, Rhodes SEV, Lopian D, Desai A, Robbins TW. Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate cortex, infralimbic cortex and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. *Behavioural Brain Research*. 2003; 156(1–2):105–119.
- Chudasama Y, Robbins TW. Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: Implications for schizophrenia. *Psychopharmacology*. 2004; 174:86–98. [PubMed: 15071717]
- Cochran SM, Kennedy M, McKerchar CE, Steward LJ, Pratt JA, Morris BJ. Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: Differential modulation by antipsychotic drugs. *Neuropsychopharmacology*. 2003; 28:265–275. [PubMed: 12589379]
- Cole BJ, Robbins TW. Forebrain norepinephrine: role in effortful but not automatic processing in the rat. *Neuropsychopharmacology*. 1992; 7:129–142. [PubMed: 1418302]
- Collings RD. Differences between ADHD inattentive and combined types on the CPT. *Journal of Psychopathological and Behavioural Assessment*. 2003; 25:177–189.
- Cornblatt BA, Keilp JG. Impaired attention, genetics, and the pathophysiology of schizophrenia. *Schizophr Bull*. 1994; 20(1):31–46. [PubMed: 8197420]
- Cornblatt BA, Malhotra AK. Impaired attention as an endophenotype for molecular genetic studies of schizophrenia. *American Journal of Medicinal Genetics*. 2001; 105:11–15.
- Coyle JT. Glutamate and schizophrenia: Beyond the dopamine hypothesis. *Cellular and Molecular Neurobiology*. 2006; 26(4–6):365–384. [PubMed: 16773445]
- Dalley JW, McGaughy J, O'Connell MT, Cardinal R, Levita L, Robbins TW. Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and non-contingent performance of a visual attentional task. *J Neuroscience*. 2001; 21:4908–4914.
- Dalley JW, Theobald DE, Pereira EAC, Li PM, Robbins TW. Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. *Psychopharmacology*. 2002; 164:329–340. [PubMed: 12424557]
- Dalley JW, Theobald DE, Bouger P, Chudasama Y, Cardinal RN, Robbins TW. Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-saporin-induced lesion of the medial prefrontal cortex. *Cereb Cortex*. 2004; 14:54–64.
- Dudchenko P, Paul B, Sarter M. Dissociation between the effects of benzodiazepine receptor agonists on behavioural vigilances and responsivity. *Psychopharmacology*. 1992; 109:203–211. [PubMed: 1365658]

- Eagle DM, Bari A, Robbins TW. The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology*. 2008; 199:439–456. [PubMed: 18542931]
- Egeland J, Johansen SN, Ueland T. Differentiating between ADHD sub-types on CCPT measures of sustained attention and vigilance. *Scandinavian Journal of Psychology*. 2009; 50:347–354. [PubMed: 19486490]
- Fattorini G, Melone M, Bragina L, Candiracci C, Cozzi A, Giampietro DEP, Torres-Ramos M, Perez-Samartin A, Matute C, Conti F. GLT-1 expression and Glu uptake in rat cerebral cortex are increased by phencyclidine. *Glia*. 2008; 56:1320–1327. [PubMed: 18615569]
- Fletcher PJ, Tenn CC, Sinyard J, Rizos Z, Kapur S. A sensitizing regimen of amphetamine impairs visual attention in the 5-choice serial reaction time test: Reversal by a D1 receptor agonist injected into the medial prefrontal cortex. *Neuropsychopharmacology*. 2007; 32:1122–1132. [PubMed: 17047670]
- Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry*. 2001; 158:1367–1377. [PubMed: 11532718]
- Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW. Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci*. 2000; 20(3):1208–1215. [PubMed: 10648725]
- Grayson B, Idris NF, Neill JC. Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the NOR task in the rat. *Behavioural Brain Research*. 2007; 184:31–38.
- Grottick AJ, Higgins GA. Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. *Behavioural Brain Research*. 2000; 117:197–208. [PubMed: 11099773]
- Grottick AJ, Higgins GA. Assessing a vigilance decrement in aged rats: effects of pre-feeding, task manipulation, and psychostimulants. *Psychopharmacology*. 2002; 164(1):33–41. [PubMed: 12373417]
- Hahn B, Shoaib M, Stolerman IP. Nicotine-induced enhancement of attention in the five-choice serial reaction time task: the influence of task demands. *Psychopharmacology*. 2002; 162:129–137. [PubMed: 12110990]
- Hahn B, Sharples CG, Wonnacott S, Shoaib M, Stolerman IP. Attentional effects of nicotine agonists in rats. *Neuropharmacology*. 2003; 44(8):1054–1067. [PubMed: 12763099]
- Hajszan T, Leranath C, Roth RH. Subchronic phencyclidine treatment decreases the number of dendritic spine synapses in the rat prefrontal cortex. *Biol Psychiatry*. 2006; 60:639–644. [PubMed: 16814748]
- Heekeren K, Daumann J, Neukirch A, Stock C, Kaeohl W, Norra C, Waberski TD, Gouzoulis-Mayfrank E. Mismatch negativity generation in the human 5HT_{2A} agonist and NMDA antagonist model of psychosis. *Psychopharmacology*. 2008; 199:77–88. [PubMed: 18488201]
- Herreo JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A. Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature*. 2008
- Ibrahim HM, Tamminga CA. Schizophrenia: Treatment targets beyond monoamine systems. *Annu Rev Pharmacol Toxicol*. 2010; 51:189–209.
- Idris NF, Repeto P, Neill JC, Large CH. Investigation of the effects of lamotrigine and clozapine in improving reversal-learning impairments induced by acute phencyclidine and D-amphetamine in the rat. *Psychopharmacology*. 2005; 179(2):336–348. [PubMed: 15645224]
- Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991; 148(10):1301–1308. [PubMed: 1654746]
- Jenkins TA, Harte MK, McKibben CE, Elliott JJ, Reynolds GP. Disturbances in social interaction occur along with pathophysiological deficits following sub-chronic phencyclidine administration in the rat. *Behav Brain Res*. 2008; 194(2):230–235. [PubMed: 18706451]
- Jentsch JD, Redmond DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH. Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. *Science*. 1997a; 277:953–955. [PubMed: 9252326]

- Jentsch JD, Tran A, Le D, Youngren KD, Roth RH. Sub-chronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. *Neuropsychopharmacology*. 1997b; 17:92–99. [PubMed: 9252984]
- Jentsch JD, Dazzi L, Chhatwal JP, Verrico CD, Roth RH. Reduced prefrontal cortical dopamine, but not acetylcholine, release in vivo after repeated, intermittent phencyclidine administration to rats. *Neurosci Lett*. 1998; 258:175–178. [PubMed: 9885959]
- Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology*. 1999; 20(3):201–225. [PubMed: 10063482]
- Jones DNC, Barnes JC, Kirkby DL, Higgins GA. Age-associated impairments in a test of attention: Evidence for involvement of cholinergic systems. *The Journal of Neuroscience*. 1995; 15(11): 7282–7292. [PubMed: 7472482]
- Karasawa J, Hashimoto K, Chaki S. d-Serine and a glycine transporter inhibitor improve MK-801 induced cognitive deficits in a novel object recognition test in rats. *Behavioural Brain Research*. 2008; 186(1):78–83. [PubMed: 17854919]
- Kern RS, Nuechterlein KH, Green MF, Baade LE, Fenton WS, Gold JM, Keefe RS, Mesholam-Gately R, Mintz J, Seidman LJ, Stover E, Marder SR. The MATRICS Consensus Cognitive Battery, part 2: co-norming and standardization. *Am J Psychiatry*. 2008; 165(2):214–220. [PubMed: 18172018]
- Krystal JH, Karper LP, Seibyl JP. Subanaesthetic effects of the non-competitive NMDA antagonist ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine response. *Arch Gen Psychiatry*. 1994; 51(3):199–214. [PubMed: 8122957]
- Leonard, JA. Anonymous Med Res Council Appl Psychol Res Unit Rep. Great Britain: 1959. 5 choice serial reaction apparatus.
- Le Pen G, Grottick AJ, Higgins GA, Moreau J. Phencyclidine exacerbates attentional deficits in a neurodevelopmental rat model of schizophrenia. *Neuropsychopharmacology*. 2003; 28:1799–1809. [PubMed: 12784101]
- Luby ED, Cohen BD, Rosenbaum F, Gottlieb J, Kelley R. Study of a new schizophrenomimetic drug, Sernyl. *Arch Neurol Psychiatry*. 1959; 81:363–369. [PubMed: 13626287]
- Mackworth JF. Vigilance, Arousal, and Habituation. *Psychological Review*. 1968; 75(4):308–322. [PubMed: 4875885]
- Marder SR, Fenton W, Youens K. Schizophrenia, IX: Cognition in schizophrenia – the MATRICS initiative. *Am J Psychiatry*. 2004; 161(1):25. [PubMed: 14702244]
- Marston HM. Analysis of cognitive function in animals, the value of SDT. *Cognitive Brain Research*. 1996; 3:269–277. [PubMed: 8806028]
- McGaughy J, Sarter M. Behavioural vigilance in rats: Task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology (Berl)*. 1995; 117(3):340–357. [PubMed: 7770610]
- McLean SL, Beck JP, Woolley ML, Neill JC. A preliminary investigation into the effects of antipsychotics on sub-chronic phencyclidine-induced deficits in attentional set-shifting in female rats. *Behavioural Brain Research*. 2008; 189(1):152–158. [PubMed: 18282619]
- McLean SL, Idris NF, Woolley ML, Neill JC. D₁-like receptor activation improves PCP-induced cognitive deficits in animal models: Implications for mechanisms of improved cognitive function in schizophrenia. *European Neuropsychopharmacology*. 2009; 19:440–450. [PubMed: 19268547]
- Mirza NR, Stolerman IP. Nicotine enhances sustained attention in the rat under specific task conditions. *Psychopharmacology*. 1998; 138:266–274. [PubMed: 9725748]
- Morgan CJ, Riccelli M, Maitland CH, Curran HV. Long-term effects of ketamine: Evidence for a persisting impairment of source memory in recreational users. *Drug Alcohol Depend*. 2004; 75:301–308. [PubMed: 15283951]
- Morgan CJ, Muetzfeldt L, Curran HV. Consequences of chronic ketamine self-administration upon neurocognitive function and psychological wellbeing: a 1-year longitudinal study. *Addiction*. 2010; 105(1):121–133. [PubMed: 19919593]
- Murai R, Noda Y, Matsui K, Kamei H, Mouri A, Matsuba K, et al. Hypofunctional glutamatergic neurotransmission in the prefrontal cortex is involved in the emotional deficit induced by repeated

- treatment with phencyclidine in mice: implications for abnormalities of glutamate release and NMDA-CaMKII signalling. *Behav Brain Res.* 2007; 180:152–160. [PubMed: 17451820]
- Murphy ER, Dalley JW, Robbins TW. Local glutamate receptor antagonism in the rat prefrontal cortex disrupts response inhibition in a visuospatial attentional task. *Psychopharmacology.* 2005; 179:99–107. [PubMed: 15678364]
- Muir JL, Robbins TW, Everitt BJ. AMPA-induced lesions of the basal forebrain: A significant role for the cortical cholinergic system in attentional function. *J Neurosci.* 1994; 14:2313–2326. [PubMed: 7512637]
- Neill JC, Barnes SA, Cook S, Grayson B, Idris N, McLean SL, Snigdha S, Rajagopal L, Harte MK. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: Focus on NMDA receptor antagonism. 2010; doi: 10.1016/j.pharmthera.2010.07.004
- Nestor PG, Faux SF, McCarley RW, Shenton ME, Sands SF. Measurement of visual sustained attention in schizophrenia using signal detection analysis and a newly developed computerized CPT task. *Schizophrenia Research.* 1990; 3:329–332. [PubMed: 2282338]
- Neuchterlein KH. Signal detection in vigilance tasks and behavioural attributes among offspring of schizophrenic mothers and among hyperactive children. *J Abnormal Psychol.* 1983; 92:4–28.
- Neuchterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. Identification of separable cognitive factors in schizophrenia. *Schizophr Res.* 2004; 72(1):29–39. [PubMed: 15531405]
- Neuchterlein KH, Green MF, Kern RS, et al. The MATRICS Consensus Cognitive Battery, Part 1: Test Selection, Reliability, and Validity. *Am J Psychiatry.* 2008; 165:203–213. [PubMed: 18172019]
- Olney JW, Faber NB. NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins and research tools for studying schizophrenia. *Neuropsychopharmacology.* 1995; 13(4):335–345. [PubMed: 8747758]
- Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. *Journal of Psychiatric Research.* 1999; 33(6):523–533. [PubMed: 10628529]
- Paine TA, Carlezon WA Jr. Effects of antipsychotic drugs on MK-801-induced attentional and motivational deficits in rats. *Neuropharmacology.* 2009; 56:788–797. [PubMed: 19705572]
- Parasuraman, R. A Bradford Book. The MIT Press; 1998. *The Attentive Brain.*
- Park SH, Kim JJ, Kim CH, Kim JH, Lee KH. Sustained attention in the context of emotional processing in patients with schizophrenia. *Psychiatry Research.* 2010; doi: 10.1016/j.psychres.2010.11.007
- Robbins, TW. Arousal and attention: Psychopharmacological and neuropsychological studies in experimental animals. In: Parasuraman, R., editor. *The attentive brain.* Cambridge: MIT Press; 1998. p. 189–220.
- Robbins TW. The 5-choice serial time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology.* 2002; 163:362–380. [PubMed: 12373437]
- Riccio CA, Reynolds CR, Lowe P, Moore JJ. The continuous performance test: A window on the neural substrates of attention. *Archives of clinical neuropsychology.* 2002; 17(3):235–272. [PubMed: 14589726]
- Sams-Dodd F. Effects of Continuous D-Amphetamine and Phencyclidine Administration on Social Behaviour, Stereotyped Behaviour, and Locomotor Activity in Rats. *Neuropsychopharmacology.* 1998; 19(1):18–25. [PubMed: 9608573]
- Sarter M. Neuronal mechanisms of the attentional dysfunctions in senile dementia and schizophrenia: two sides of the same coin? *Psychopharmacology.* 1994; 114:539–550. [PubMed: 7855215]
- Snedecor, GW., Cochran, WG. *Statistical Methods.* Eighth. Iowa State University Press; Ames, Iowa: 1989. p. 226–228.
- Snigdha S, Neill JC. Efficacy of antipsychotics to reverse phencyclidine-induced social interaction deficits in female rats – A preliminary investigation. *Behavioural Brain Research.* 2008; 187:489–494. [PubMed: 18037171]
- Snigdha S, Neill JC, McLean SL, Shemar GK, Cruise L, Shahid M, Henry B. Phencyclidine (PCP)-induced disruption in cognitive performance is gender-specific and associated with a reduction in brain-derived neurotrophic factor (BDNF) in specific regions of the female brain. *J Mol Neurosci.* 2010; 18doi: 10.1007/s12031-010-9447-5

- Spratt C, Marston HM, Sharkey J, Kelly JS. Comparison of rat and mouse attentional performance in the 5-choice serial reaction time task. *BNA*. 2000; 17:49.03.
- Stolerman IP, Mirza NR, Hahn B, Shoaib M. Nicotine in an animal model of attention. *European Journal of Pharmacology*. 2000; 393:147–154. [PubMed: 10771008]
- Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, Javitt DC. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers. *Arch Gen Psychiatry*. 2000; 57:1139–1147. [PubMed: 11115327]
- Wang Q, Chan R, Sun J, Yao J, Deng W, Sun X, Liu X, Sham PC, Ma X, Meng H, Murray RM, Collier DA, Li T. Reaction time of the continuous performance test is an endophenotypic marker for schizophrenia: A study of first-episode neuroleptic-naïve schizophrenia, their non-psychotic first-degree relatives and healthy population control. *Schizophrenia Research*. 2007; 89(1–3):293–298. [PubMed: 17055704]
- Wilkinson RT. Interaction of noise with knowledge of results and sleep deprivation. *J Exp Psychol*. 1963; 66:332–337. [PubMed: 14051850]
- Winstanely CA, Chudasama Y, Dalley JW, Theobald DEH, Glennon JC, Robbins TW. Intra-prefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. *Psychopharmacology*. 2003; 167(3):304–314. [PubMed: 12677356]
- Young JW, Crawford N, Kelly JS, Kerr LE, Marston HM, et al. Impaired attention is central to the cognitive deficit observed in alpha 7 deficient mice. *Eur Neuropsychopharmacology*. 2007; 17:145–155.
- Young JW, Powell SB, Risbrough V, Marston HM, Geyer MA. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacology and Therapeutics*. 2009a; 122:150–202. [PubMed: 19269307]
- Young JW, Light GA, Marston HM, Sharp R, Geyer MA. The 5-Choice performance test: Evidence for a translational test of vigilance for mice. *PLoS ONE*. 2009b; 4:e4227. [PubMed: 19156216]
- Young JW, Powell SB, Scott CN, Zhou X, Geyer MA. Reduced Dopamine D4 receptor expression as a mouse model of ADHD: Evidence of impaired vigilance in the 5-choice continuous performance test. (submitted to *Psychopharmacology*).

Fig 1.

Performance displayed over the duration of the session following an acute behavioural challenge consisting of introducing a variable SD. T1 – T3 refers to the trial banks, each consisting of 40 trials. Data were expressed as observed mean $\pm$ SEM and analysed by two-way repeated measures ANOVA (Trial as within-subjects factor and Treatment as between-subjects factor) followed by Planned Comparisons on the predicted means. # denotes $p < 0.05$ compared to vehicle (2.5 mg/kg PCP). * denotes $p < 0.05$, ** denotes $p < 0.01$, *** denotes $p < 0.001$ compared to vehicle (5 mg/kg PCP).

Fig 2. Sensitivity Index displayed for each individual stimulus durations presented throughout the session. Data displayed as observed mean $\pm$ SEM and analysed by two-way repeated measures ANOVA (SD as within-subjects factor and Treatment as between-subjects factor) followed by Planned Comparisons on the predicted means. The effect of SD on SI was analysed specifically for control animals via one-way repeated measures ANOVA (SD as within-subjects factor) followed by Planned Comparisons. * denotes SI for 0.25 s SD significantly reduced compared to 1 s SD.

Fig 3.

Performance displayed over the duration of the session that was extended to 256 trials. T1 – T4 refers to the trial banks, each consisting of 64 trials. Data were expressed as observed mean $\pm$ SEM and analysed by two-way repeated measures ANOVA (Trial as within-subjects factor and Treatment as between-subjects factor) followed by Planned Comparisons on the predicted means. # denotes $p < 0.05$ and ## denotes $p < 0.01$ compared to vehicle (2.5 mg/kg PCP). * denotes $p < 0.05$ and ** denotes $p < 0.01$, compared to vehicle (5 mg/kg PCP).

Fig 4.

Sensitivity Index (SI) of control animals following the extended session challenge. T1 – T4 refers to the trial banks each consisting of 64 trials. Data were expressed as observed mean $\pm$ SEM and analysed by one-way repeated measures (Trial as within-subjects factor) ANOVA followed by Planned Comparisons. * denotes $p < 0.05$ Trial bank 2 (65 – 128) significantly greater than Trial bank 4 (193 – 256).

Table 1

Description of the behavioural measures used in the 5C-CPT

Measure & Description
Accuracy: Correct responses/(correct + incorrect)*100 – a measure of selective attention
Percent Correct: Correct responses/total number of trials*100 – a measure of overall performance
Percent omission: Omissions/(correct + incorrect + omissions) *100 – a measure of sustained attention/motivation
Correct Latency: Time taken to make a correct response to assess psychomotor speed
Incorrect Latency: Time taken to make an incorrect response to assess general response speed
Magazine Latency: Time taken to collect food reward to assess motivational state
Total trials: Number of trials completed within the session
Time out responses: Repeat responses during the time out period
Premature responses: Inappropriate responses made within the ITI period to assess motor impulsivity
Perseverative responses: Inappropriate repeat response following a correct response
Correct Rejections: Correctly withheld/(correct rejections + false alarms)*100 – a measure of correct rejection of no-go trials
Hit rate, p[HR]: The proportion of target trials correctly detected
False alarm rate, p[FA]: The proportion of non-target trials incorrectly responded to: to assess response disinhibition
Sensitivity index, SI: Non-parametric measure of the ability to discriminate between target and non-target trials – a measure of vigilance
Responsivity index, RI: Non-parametric measure of the response bias or strategy of the animal

Further information concerning the calculations of these measures are described in Amitai et al. 2007 and Young et al. 2009b

Table 2

Effect of Sub-Chronic PCP on overall performance in 5C-CPT with stable baseline

Measure	Vehicle	2.5 mg/kg PCP	5.0 mg/kg PCP
Accuracy (%)	94.52 ± 1.41	93.68 ± 1.13	93.56 ± 1.49
Percent Correct	78.63 ± 3.40	74.56 ± 3.07	73.78 ± 3.56
Percent Omission	17.03 ± 2.84	20.56 ± 2.71	21.32 ± 3.26
Correct Latency	0.67 ± 0.04	0.64 ± 0.03	0.68 ± 0.05
Incorrect Latency	0.82 ± 0.12	0.86 ± 0.14	0.91 ± 0.10
Magazine Latency	1.22 ± 0.08	1.17 ± 0.05	1.26 ± 0.06
Perseverative Response	1.82 ± 0.85	2.00 ± 0.66	4.60 ± 2.12
Premature Response	6.55 ± 1.86	10.09 ± 1.80	11.90 ± 2.49
Time Out Response	8.18 ± 1.62	11.00 ± 3.10	14.40 ± 4.14
p[HR]	0.79 ± 0.03	0.75 ± 0.03	0.74 ± 0.04
p[FA]	0.26 ± 0.04	0.31 ± 0.05	0.34 ± 0.06
Sensitivity Index	0.54 ± 0.06	0.45 ± 0.04	0.42 ± 0.06
Responsivity Index	0.06 ± 0.07	0.06 ± 0.08	0.06 ± 0.09

Measures are shown as observed mean ± SEM. PCP treatment resulted in no significant effect under these conditions

Table 3

Effect of Sub-Chronic PCP on overall performance in 5C-CPT using a variable SD

Measure	Vehicle	2.5 mg/kg PCP	5.0 mg/kg PCP
Accuracy (%)	88.15 ± 1.57	85.23 ± 2.79	81.43 ± 2.55
Percent Correct	67.42 ± 2.93	62.55 ± 2.72	53.79 ± 2.79 **
Percent Omission	23.58 ± 2.86	26.65 ± 2.04	33.79 ± 2.79 *
Correct Latency	0.68 ± 0.03	0.58 ± 0.04	0.63 ± 0.06
Incorrect Latency	0.93 ± 0.07	0.81 ± 0.06	0.99 ± 0.11
Magazine Latency	1.18 ± 0.06	1.14 ± 0.06	1.26 ± 0.08
Perseverative Response	1.00 ± 0.54	2.27 ± 1.23	3.50 ± 1.59
Premature Response	3.82 ± 0.98	7.27 ± 1.52	9.60 ± 2.86
Time Out Response	8.09 ± 1.33	12.00 ± 2.28	16.70 ± 2.58 *
p[HR]	0.67 ± 0.03	0.63 ± 0.03	0.54 ± 0.03 **
p[FA]	0.18 ± 0.04	0.27 ± 0.03	0.32 ± 0.06 *
Sensitivity Index	0.52 ± 0.05	0.36 ± 0.04*	0.24 ± 0.07 ***
Responsivity Index	-0.21 ± 0.07	-0.12 ± 0.05	-0.17 ± 0.07

Measures are shown as observed mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05, **p<0.01) compared to the vehicle group following Planned Comparisons.

Table 4

Within-session alteration of strategy in control animals following vSD challenge

Trial Number	0 – 40	41 – 80	81 – 120
Responsivity Index	-0.11 ± 0.09	-0.24 ± 0.09	$-0.37 \pm 0.08^{**}$

Measures are shown as mean $\pm$ SEM. A more conservative response strategy was adopted in control animals as the session progressed, an effect that was absent in PCP treated animals.

^{**} denotes $p < 0.01$ when compared to trial number 0 - 40

Table 5

Effect of Sub-Chronic PCP on overall performance in 5C-CPT following an extended session

Measure	Vehicle	2.5 mg/kg PCP	5.0 mg/kg PCP
Accuracy (%)	93.85 ± 1.13	92.03 ± 1.45	90.38 ± 1.82
Percent Correct	70.17 ± 3.81	68.69 ± 3.14	61.50 ± 4.57
Percent Omission	25.28 ± 3.87	25.51 ± 2.86	32.18 ± 4.49
Correct Latency	0.71 ± 0.03	0.59 ± 0.03 *	0.63 ± 0.04
Incorrect Latency	1.13 ± 0.08	1.12 ± 0.12	1.27 ± 0.12
Magazine Latency	1.17 ± 0.05	1.13 ± 0.05	1.28 ± 0.07
Perseverative Response	2.00 ± 0.56	5.45 ± 1.71	7.10 ± 2.16 *
Premature Response	6.73 ± 1.44	13.55 ± 2.80	16.90 ± 5.10
Time Out Response	12.45 ± 1.29	20.55 ± 3.49	32.30 ± 8.51 *
p[HR]	0.70 ± 0.04	0.69 ± 0.03	0.61 ± 0.05
p[FA]	0.16 ± 0.04	0.28 ± 0.03 *	0.28 ± 0.04 *
Sensitivity Index	0.57 ± 0.04	0.42 ± 0.03 *	0.36 ± 0.06 **
Responsivity Index	-0.19 ± 0.10	-0.04 ± 0.06	-0.12 ± 0.07

Measures are shown as observed mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05, **p<0.01) compared to the vehicle group.