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Phencyclidine dose optimisation for induction of spatial learning and memory deficits related to schizophrenia in C57BL/6 mice

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Abstract: Phencyclidine (PCP) has been used to model cognitive deficits related to schizophrenia in rats and mice. However, the model in mice is not consistent in terms of the PCP effective dose reported. Furthermore, most of the previous studies in mice excluded the presence of drug washout period in the regime. Thus, we aimed to optimize the dose of PCP in producing robust cognitive deficits by implementing it in a PCP regime which incorporates a drug washout period. The regimen used was 7 days' daily injection of PCP or saline for treatment and vehicle groups, respectively; followed by 24 h drug washout period. After the washout period, the test mice were tested in water maze (5 days of acquisition + 1 day of probe trial) for assessment of spatial learning and memory. Initially, we investigated the effect of PCP at 2mg/kg, however, no apparent impairment in spatial learning and memory was observed. Subsequently, we examined the effect of higher doses of PCP at 5, 10 and 20 mg/kg. We found that the PCP at 10 mg/kg produced a significant increase in "latency to reach the platform" during the acquisition days and a significant increase in "latency of first entry to previous platform" during the probe day. There was no significant change observed in "swim speed" during the test days. Thus, we concluded that PCP at 10 mg/kg produced robust deficits in spatial learning and memory without being confounded by motor disturbances.

Key words: learning, maze, memory, phencyclidine, schizophrenia

Introduction

Schizophrenia is a complex mental disorder which is characterised by positive, negative and cognitive deficits symptoms. The occurrence of cognitive deficits is believed to be a core feature of schizophrenia. There are multiple domains of cognitive function that are disturbed

in schizophrenia including working memory, attention, learning and memory, and social cognition [11].

Phencyclidine (PCP), also known by its street name 'angel dust', is a non-competitive N-methyl-D-aspartate (NMDA)/glutamate receptor antagonist [12]. The administration of PCP into healthy human subjects induced schizophrenic-like symptoms such as hallucinations and

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delusions, negative symptoms and a range of cognitive deficits [10]. The ability to mirror a more complete range of schizophrenic symptoms makes PCP, perhaps, a better pharmacological model of schizophrenia than amphetamine which can only mirror the positive symptoms of the disorder by elevating extracellular levels of dopamine (DA) and prolonging its signalling in the striatum [7]. These effects of PCP are not only exclusive to human but also replicable in rodents [1] and primates [7].

In essence, PCP has been used to model cognitive deficits related to schizophrenic symptoms in the rodent. However, the PCP treatment regime adopted appears to be different in rats and mice. In the former, for example, the most commonly used regime was twice daily injections of PCP (2–5 mg/kg) for seven days followed by seven days of drug washout period; herein described as sub-chronic [9]. The drug washout period is important to minimise a direct drug effect or drug withdrawal effect in influencing the behavioural test [1, 8]. Furthermore, a sub-chronic treatment of PCP with the presence of a drug washout period produces more sustainable cognitive deficits with reasonably similar neuropathological and abnormal behaviour of schizophrenia [12]. However, a previous study which utilized mice failed to demonstrate significant cognitive deficits induced by similar PCP regime used for rats (twice daily injections of 5 mg/kg PCP for seven days followed by seven days of washout period) and this was suggested to be due to the species difference [5]. Moreover, in our unpublished work, similar PCP regime but also at higher doses (5, 9 and 13 mg/kg, intraperitoneal (i.p.)), did not produce any significant cognitive deficits in mice, which suggested that a drug washout period for 7 days may be too long for mice.

While the commonly used PCP regime in rats contained a drug washout period, the one commonly used in mice contained a pre-treatment period with low doses of PCP in order to cause tolerance to the adverse-effects of PCP; and followed by acute PCP injections prior to the behavioural task [13]. However, cognitive deficits produced from this commonly used PCP regime in mice may potentially be confounded from the direct effect of the acute PCP, rather than from biological changes resembling the pathological brain of schizophrenia patients. Moreover, previous studies also reported many different effective doses of PCP in impairing cognitive functions in mice. It was suggested that the dose of PCP must be enough to cause cognitive deficits without caus-

ing unwanted adverse-effects such as ataxia, intense locomotor activity, motor disturbance and neurological dysfunction to be considered as an effective dose [4, 14]. Nevertheless, a study did report that the PCP dose at 2.0 mg/kg in a regime of 7 days daily subcutaneous, followed by 24 h of washout period produced a consistent impairment in spatial learning and working memory performance in the Morris water maze task without any apparent motor deficits [4]. However, we failed to replicate the results of Beraki *et al.* (2009) [4] using the same PCP regime and dose.

In the present study, our objective was to find an effective dose of PCP in the subchronic drug regime which incorporates a washout period. Thus, we implemented the same PCP regime by Beraki *et al.* (2009) [4] but at higher doses of PCP (5, 10 and 20 mg/kg) to model cognitive deficits related to schizophrenia in mice. We compared the performance of the vehicle control and the PCP groups in the Morris water maze task to evaluate the spatial learning and memory function. In the discussion, we also compared and discussed the results from the present and previous studies that had been carried out in mice and rats.

Materials and Methods

Experimental animals

The present study protocol was approved by the UiTM CARE (Committee on Animal Research and Ethics), Universiti Teknologi MARA (UiTM), Selangor, Puncak Alam, Malaysia. Male C57BL/6 mice (20–25 g body weight) were purchased from Monash University, Malaysia. Upon delivery to UiTM, the animals were housed in individually ventilated cages (IVC) typically in a group of 3–4 mice, and were maintained at a standard room temperature (20–22°C) and a relative humidity of 50–70% in normal light and dark cycles of 12 h interval (Lights on at 7AM) for one week. Animals were provided food (commercial mice chow) and drink *ad libitum* during the entire study period. The first set of animals was used to study the effect of 2 mg/kg of PCP and another set of animals was used to study the effect of higher doses of PCP.

Camera and software used

All experiments performed in this study were recorded by a camera mounted on the ceiling above the apparatus and connected to the computer in the test room.

The ANY-maze software was used for recording, animal tracking and analysing the experimental data.

Drug treatment

Phencyclidine hydrochloride (Sigma-Aldrich) was dissolved in sterile normal saline (0.9%w/v NaCl). The vehicle control group received an intraperitoneal injection of sterile normal saline whereas PCP-treated groups received an intraperitoneal injection at doses of 2, 5, 10, and 20 mg/kg for seven consecutive days at a constant volume of injection 10 ml/kg. All animals were given one day washout period after the last injection before being tested in a Morris water maze task. The body-weight of mice on the first and the fifth day of injection were recorded.

Morris water maze (MWM)

The water maze apparatus was an open white circular shaped pool with a smooth surface on the bottom and the side-wall. The pool was in the size of 179 cm in diameter with a square shaped escape platform (9.5 cm in length), making the search area to target ratio of 279:1. The pool was filled with tap water and stained with a non-toxic white chalk to make it opaque. The escape platform was submerged 0.5 cm below the water surface. The room lighting was indirect to avoid reflection on the water surface. Four intentional cues in the form of Star, Circle, Rectangle and Triangle were glued to the side-wall of the pool at 3 cm above the water level. The pool was divided into four equal sized of the quadrants (North-East, East-South, South-West and West-North) using the software. The hidden platform was placed in the middle of the East-South zone.

The experimental animals were acclimatised inside the test room for a day before the experiment. The experimental protocol of MWM test was adapted from a previous study [15]. Briefly, the MWM test consists of two phases; spatial learning (acquisition) and spatial memory (probe). The MWM test was started approximately 24 h after the last treatment. During the acquisition phase, the mice were trained for 5 days, with 3 trials per day, to find the hidden platform. Each trial was set for only 90 s with an inter-trial gap of 4–6 min for each mouse to rest. Latency to reach the platform, path-length, swim speed, and latency of first entry to quadrant where the platform located were recorded and analysed.

After 5 days of acquisition, the probe day was started. In this phase, the hidden platform was removed from the

pool and the spatial memory was measured. The mice were released at the opposite quadrant from the one where the hidden platform was previously placed. Only one trial was performed over 60 s in this phase. During the 60 s, the previous platform entries, path efficiency to first platform entry, latency of first entry to previous platform, and latency of first entry to the quadrant where the platform was previously located were recorded and analysed.

Statistical analysis

Data from the bodyweight changes during PCP treatment and the Morris water maze for spatial learning were analysed by two-way repeated measures analysis of variance (ANOVA) with DAY as within-subjects factor and GROUP as between-subjects factors, followed by Bonferroni correction when appropriate. The Greenhouse-Geisser or Huynh-Feldt correction was used when assumption of sphericity was not met. Results obtained during probe trials for the 2 mg/kg PCP study was analysed separately by two-tail *t*-test assuming equal variances with vehicle as the control group. Results obtained during probe trials for the 5, 10 and 20 mg/kg of PCP study, however, were analysed by one-way ANOVA and Kruskal-Wallis where appropriate. Microsoft Excel 2007 and SPSS statistical software version 16.0 were used for all statistical analyses. The $P < 0.05$ was accepted as a significant value for all data analysis. All the data were expressed as mean $\pm$ SEM.

Results

PCP at 2 mg/kg failed to impair spatial learning and memory

During the acquisition days, two-way repeated measures ANOVA revealed that there was no significant interaction between DAY and GROUP factors for all the three parameters, namely latency to reach the platform ($F_{4,56}=0.221$, $P=0.926$; Fig. 1a), path-length ($F_{4,56}=0.167$, $P=0.954$; Fig. 1b) and latency of first entry to quadrant where the platform located ($F_{4,56}=1.872$, $P=0.128$). Test of within-subjects effect revealed significant differences between days of test, but no significant difference was detected for between-subject effects of group in all three parameters.

For the swim speed parameter, there was no significant interaction between DAY and GROUP factors. Test of within-subjects effects of day showed no significant dif-

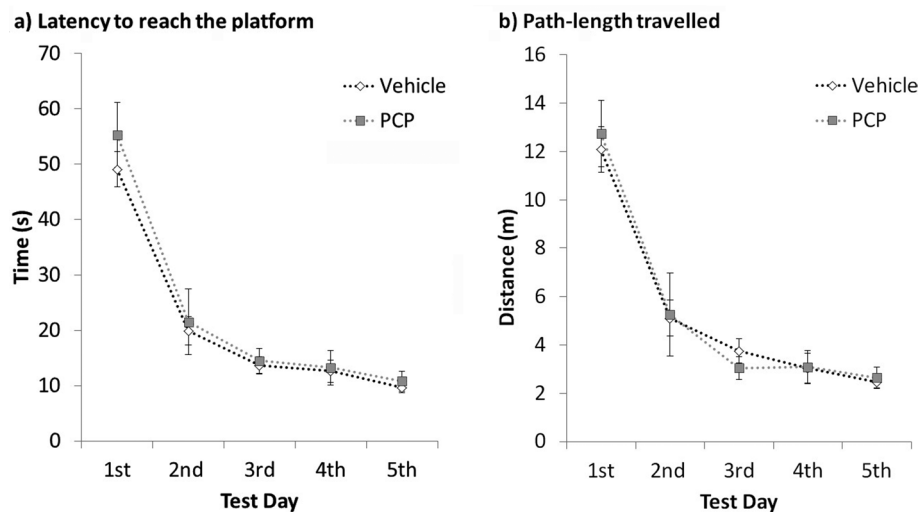


Fig. 1. The effect of 2.0mg/kg dose of PCP on the spatial learning memory during the 5 days of Morris water maze test. a) Time taken by the vehicle control and PCP groups to find the hidden platform. b) Total distance travelled by the control and PCP groups to reach the hidden platform. Data are mean $\pm$ SEM. $n=8$ per treatment.

ference in swim speed during the five days of acquisition. In addition, there was also no significant difference in swim speed between-subjects effects of group. Results of within-subjects effects of day indicate that the swim speed of vehicle and PCP groups did not differ significantly between each other and did not also significantly differ between days of test.

During the probe trial, there was no significant difference between vehicle control and PCP (2 mg/kg, i.p.) treated groups in previous platform entries ($t_{14}=1.374$, $P=0.191$), path efficiency to first platform entry ($t_{14}=-1.034$, $P=0.319$), latency of first entry to previous platform ($t_{14}=-1.227$, $P=0.240$) and latency of first entry to the quadrant where the platform was previously located ($t_{14}=-1.949$, $P=0.072$).

PCP at 10 mg/kg produced robust impairment in spatial learning and memory

There was no significant change in bodyweight of vehicle control and PCP groups during the PCP treatment ($F_{1,14}=2.395$, $P=0.144$), indicating that PCP at 10 mg/kg did not produce any effect on bodyweight status. During the acquisition days of the Morris water maze, two-way repeated measures ANOVA revealed that there was no significant interaction between DAY and GROUP factors in all three parameters measured. Test of within-subjects effects of day showed a significant difference in latency to reach the platform, path-length, and latency of first

entry to quadrant where the platform located during the five days of acquisition. Test of between-subjects effects revealed a significant difference in parameters of latency to reach the platform ($F_{3,24}=7.939$, $P=0.001$; Fig. 2a), path-length ($F_{3,24}=4.302$, $P=0.015$; Fig. 2b), and latency of first entry to quadrant where the platform located ($F_{3,24}=5.086$, $P=0.007$; Fig. 2c). There was no significant difference between PCP treatment groups in all the three parameters. Pairwise comparison with Bonferroni correction between PCP groups versus vehicle control group is summarized in Table 1. Only PCP at 10 mg/kg shows significant difference in all the three parameters as compared with the vehicle control.

For the swim speed parameter during acquisition days, there was no significant interaction between DAY and GROUP factors. The test of within-subjects effects revealed no significant difference during the five days of acquisition, and between-subjects effects also revealed no significant difference among the 4 test groups.

During the probe trial, there was no significant difference between the vehicle and all the three PCP groups in previous platform entries (Fig. 3a) and path-efficiency to first platform entry. Nonetheless, the one-way ANOVA revealed that there was a statistically significant difference between-groups effects ($F_{3,19}=6.639$, $P=0.003$) in latency for first entry to previous platform (Fig. 3c). The Bonferroni *post hoc* tests for multiple comparison showed that PCP at 10 mg/kg was significantly higher

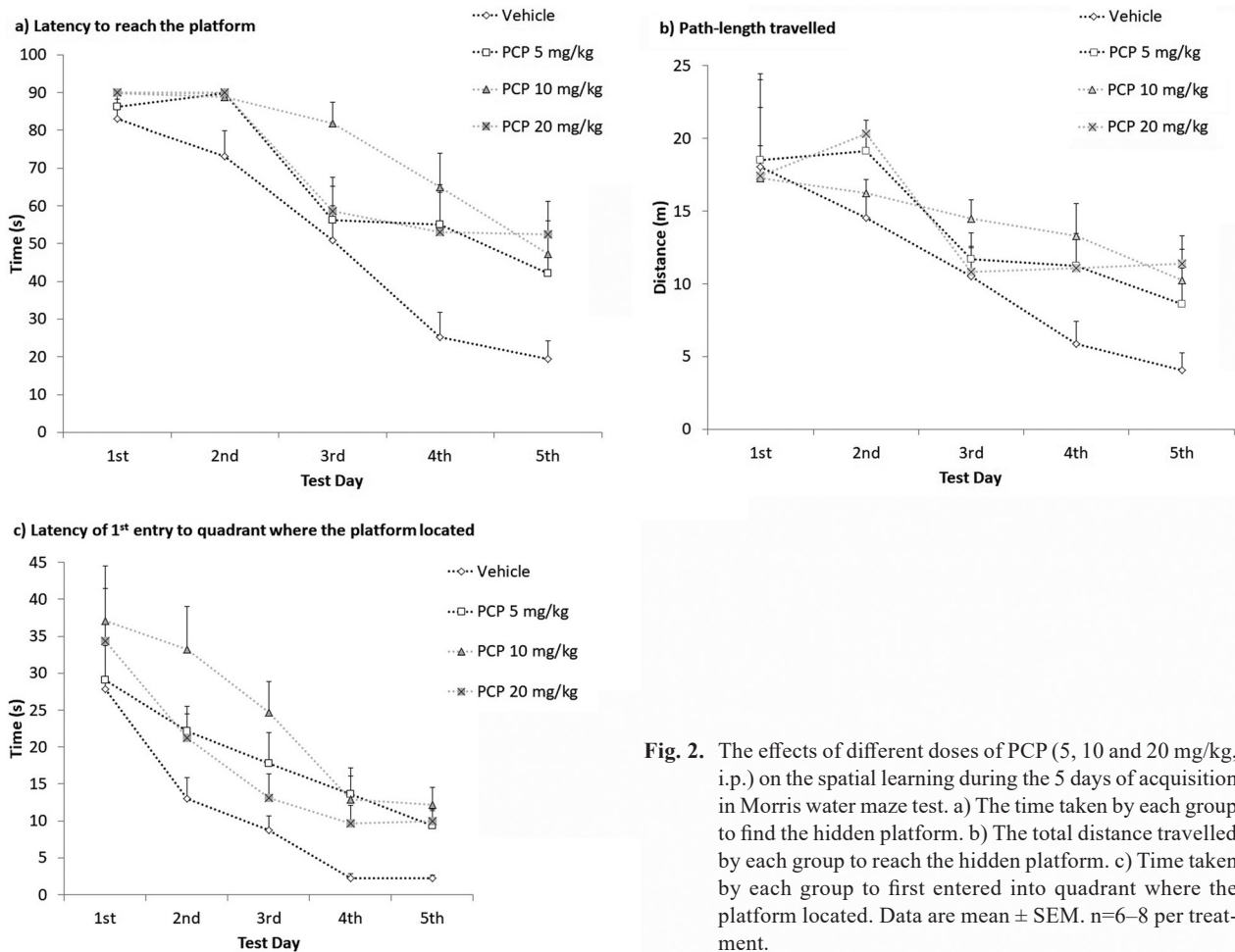


Fig. 2. The effects of different doses of PCP (5, 10 and 20 mg/kg, i.p.) on the spatial learning during the 5 days of acquisition in Morris water maze test. a) The time taken by each group to find the hidden platform. b) The total distance travelled by each group to reach the hidden platform. c) Time taken by each group to first entered into quadrant where the platform located. Data are mean $\pm$ SEM. $n=6-8$ per treatment.

Table 1. Pairwise comparison between PCP at 5.0, 10, and 20 mg/kg versus vehicle group during the 5 days of acquisition in the Morris water maze task

Group of comparison		Latency to reach the platform (s)		Path-length (m)		Latency of 1st entry to quadrant where the platform located (s)	
		*Mean Difference	**P-value	*Mean Difference	**P-value	*Mean Difference	**P-value
Vehicle	PCP 5.0 mg/kg	-15.51	0.055	-3.32	0.096	-7.634	0.256
	PCP 10 mg/kg	-24.231	0.001	-3.702	0.036	-13.247	0.004
	PCP 20 mg/kg	-18.432	0.011	-3.604	0.043	-6.931	0.322

Only PCP at 10 mg/kg shows significant difference in all the three parameters measured: latency to the reach the platform, path-length, and latency of 1st entry to quadrant where the platform located. *mean difference was calculated by subtracting the mean of PCP group from vehicle group. **P-value adjusted for multiple comparisons Bonferroni correction.

in latency for first entry to previous platform as compared to vehicle ($P=0.002$). For the latency of first entry to the quadrant where the platform was previously located, the Levene's test for homogeneity of variances showed a violation ($F_{3,24}=6.173$, $P<0.003$). Thus, a non-parametric test was preferred to analyse this parameter. The Kruskal-Wallis test revealed that there was a sig-

nificant difference between groups of test in the parameter of latency of first entry to the quadrant where the platform was previously located ($\chi^2_3=9.922$, $P=0.019$). Multiple comparisons were then conducted by two-tailed Mann-Whitney test, and corrected by Bonferroni *post hoc*. It was revealed that PCP at 5 and 10 mg/kg significantly increased the latency of first entry to the quad-

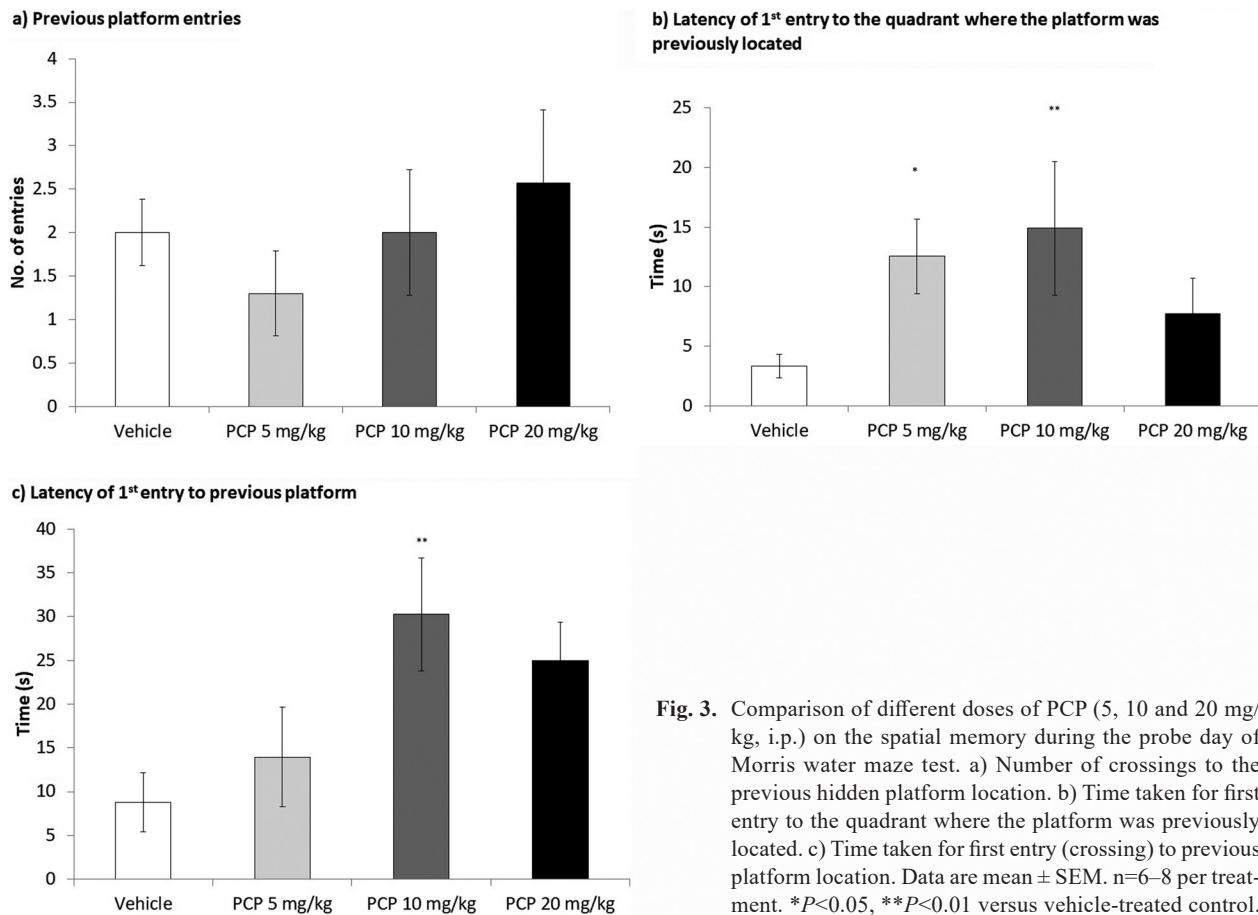


Fig. 3. Comparison of different doses of PCP (5, 10 and 20 mg/kg, i.p.) on the spatial memory during the probe day of Morris water maze test. a) Number of crossings to the previous hidden platform location. b) Time taken for first entry to the quadrant where the platform was previously located. c) Time taken for first entry (crossing) to previous platform location. Data are mean $\pm$ SEM. $n=6-8$ per treatment. * $P<0.05$, ** $P<0.01$ versus vehicle-treated control.

rant where the platform was previously located as compared to the vehicle group (5 mg/kg PCP, $U=7$, $P=0.028$; 10 mg/kg PCP, $U=3$, $P=0.004$; Fig. 3b). However, following Bonferroni correction for 6 comparisons, only PCP at 10 mg/kg remained significant.

Discussion

In the present study, we examined the effect of different doses of PCP on spatial learning and memory in C57BL/6 mice using Morris water maze test. The PCP regime utilized in this study was closest to the regime commonly used in rat studies which included a drug washout period. The present study revealed that the PCP at 10 mg/kg, i.p., produced a robust impairment of spatial learning and memory in Morris water maze task as compared to the PCP at doses of 2, 5 or even at 20 mg/kg. Our results are neither confounded by the impairment of motor activity as indicated by no significant changes in the “swim speed” during acquisition and probe trials,

nor by lack of motivation to escape as indicated by significant changes in the “path-length to reach platform” during the acquisition trial.

The number of studies related to cognitive deficits model of schizophrenia in mice and rats is disproportionate, in which only few studies have established the genetic model in rats, and few studies have established the pharmacological model in mouse [16]. Nonetheless, the mouse pharmacological model is currently in high demand because of a rapid development that has occurred in the mouse genetic modification technology. Few genes which have been shown to be associated with schizophrenia such as disrupted-in-schizophrenia 1 (*DISC1*), neuregulin 1 (*NRG1*), and dysbindin (*DTNBP1*) genes have been extensively studied using genetic models of knock-out mice approach. These mice have shown some resemblance to schizophrenia symptoms in terms of pathological and behavioural alterations, but are not able to replicate the full syndrome of schizophrenia which is to be expected from single gene manipulations [9]. It

would be interesting to see a combined approach of the genetic and pharmacological model in simulating schizophrenic symptoms; however, there is firstly, a need to verify the existing pharmacological models in mice with the purpose of making it more replicable across studies.

Many of the previous studies which examined the effect of PCP on spatial learning and memory by using water maze in mice were mainly focused on the low doses of PCP (0.5–2.5 mg/kg). One of the arguments that have been used to justify this approach was to exclude the potential neurotoxic effect of PCP administration [4]. Nonetheless, there is no evidence yet which associates the neurotoxicity effect of PCP with changes in animal behaviour. Moreover, the effective PCP dose which is commonly used in the rats were around 2–5 mg/kg; therefore, the equivalent surface area dose when extrapolation was made from the rat to the mouse is estimated to be around 4–10 mg/kg (the conversion dose factor from rats to mice is 2) [6]. We summarize the details of previous published reports using PCP to induce schizophrenic-like symptoms in rats and mice in Table 2. More comprehensive comparison between mice and rats in modelling cognitive deficits in schizophrenia can be referred to Young *et al.* (2012) [16].

In a previous study, Podhorna *et al.* (2005) [13] reported that PCP at 2.5 mg/kg impaired spatial learning in C57BL/6 mice during the acquisition days of water maze, but did not affect spatial memory during the probe trial. The authors suggested that 10 day pre-treatment of 2.5 mg/kg of PCP was needed to induce drug tolerance in mice, and PCP should be given prior to the first trial on each day of acquisition in order to impair spatial learning [13]. In another study, the effect of 0.25–4 mg/kg of PCP was studied in C57BL/6 mice using repeated PCP injections for 12 days; in which during the last 5 days, the PCP treatment was followed by water maze training after 15 min [3]. The authors found that only 0.5 mg/kg PCP produced spatial learning and memory deficits without any apparent motor disturbance. Moreover, in a separate part of their study that investigated the effect of 0.5, 1, and 2.0 mg/kg PCP, it was concluded that PCP at 2.0 mg/kg produced the most robust spatial learning and memory deficits in C57BL/6 mice when compared to the other two doses [4]. Nevertheless, this has been argued by Young *et al.* in his review, as the deficits reported by Beraki (2009) seems to be confounded by floating behaviour rather than by impaired memory [16]. We replicated the same PCP regime of Beraki

(2009) [4]; however, the present study failed to show the same memory impairment caused by PCP at 2.0 mg/kg using the same PCP regime. The discrepancies between the present study and the previous report could be due to differences in the search area to target ratio; the present study used 279:1 of search area to target ratio, while the study conducted by Beraki (2009) [4] used a smaller version of 144:1. The search area to target ratio is important to determine the difficulty of the learning task to the animal; in which the bigger the ratio, the more difficult the task is [15]. It is to be noted that both the present and the previous studies showed that the vehicle group reached asymptomatic performance on the fifth acquisition day, which indicates that both the vehicle groups are able to learn how to find the platform.

In addition, the present study also reported that PCP at 20 mg/kg produced less robust memory deficits compared than 10 mg/kg, but also more robust than 2 and 5 mg/kg of PCP. Thus, it is suggested that instead of linear-shaped dose-effect relationship, the effect of PCP doses on spatial learning and memory is more likely to be an inverted U-shaped dose-effect relationship. However, this relationship is limited to the ranges of PCP observed in the present study and to the subchronic regime only. Besides, the present study was not purposely designed to investigate such a relationship. Nonetheless, the inverted U dose-effect curve has been reported to be frequently observed in many pharmacological or non-pharmacological compounds including the effects of NMDA antagonist on learning and memory [2]. Our present results appear to support this hypothesis.

We conclude that PCP at 10 mg/kg using the regime of 7 days daily injections followed by 24 h washout period was able to produce robust deficits in spatial learning and memory which resemble cognitive deficits related to schizophrenia. In the regime used, the washout period of 24 h has prevented motor disturbance induced by PCP, indicated by no changes in the swim speed. Our results were not confounded by lack of motivation or floating behaviour as indicated by significant changes in path-length during the acquisition trial. Nevertheless, our results are limited to spatial memory and learning as assessed by the water maze test. Whether the same PCP dose and regime will work in other cognitive behavioural tasks of mice such as working memory and social interaction tasks would require further investigations.

Table 2. Comparison between the present study and previous studies which utilized PCP-induced cognitive deficits related to schizophrenia in term of PCP dose and regime used, species, gender, behavioural test, and the deficits produced

References	Species/Strain/Sex	PCP regime	Washout period/ period between the last injection and behavioural test	Effective PCP dose (PCP dose investi- gated, if have)	Behavioural Test	Cognitive domain deficits
Present Study	Mice/C57BL/6/ ♂	Sub-chronic: 7 days daily injections	1 day	10 mg/kg (2, 5, 10 and 20 mg/kg)	MWM	Deficits in spatial learning and memory
(Beraki, <i>et al.</i> , 2009)	Mice/C57BL/6/ ♂	Sub-chronic: 7 days daily injections	1 day	2 mg/kg (0.5, 1 and 2 mg/kg)	MWM	Deficits in spatial learning and memory
(Beraki, <i>et al.</i> , 2008)	Mice/C57BL/6/ ♂	Repeated + acute: 12 days daily injections	Test was performed during the last 5 days of injections	0.5 mg/kg (0.5, 1, 2, and 4 mg/kg)	MWM	Deficits in spatial learning and memory
(Podhorna and Didriksen, 2005)	Mice/C57BL/6/♂	Repeated + acute: 14 days daily injections	Test was performed during the last 4 days of injections	2.5 mg/kg (1.3, 2.5, and 5 mg/kg)	MWM	Deficits in spatial learning and memory
	Rats/Wistar/♂	Repeated + acute: 7 days daily injection		1.3 mg/kg (1.3 and 2.5 mg/kg)	MWM	Deficits in spatial learning and memory
(Ihalainen, <i>et al.</i> , 2016)	Rats/Lister hooded/♂ Rats/Wistar/♂	Repeated + acute: 8 days daily injection	Test was performed during the last 5 days of injection	2.0 mg/kg (1.3, 1.6 and 2.0 mg/kg)	MWM	Deficits in spatial learning and memory
(Hashimoto, <i>et al.</i> , 2008)	Mice/ICR/♂	Intermittent: 10 days daily injection	16 days	10 mg/kg	NORT	Deficits in visual recognition memory
(Brigman, <i>et al.</i> , 2009)	Mice/C57BL/6J/♂	Sub-chronic: 7 days twice daily injections	7 days	5 mg/kg	Sociability and social novelty preference Pairwise visual discrimination and reversal learning	Partial deficits in sociability and social recognition memory No deficits in executive function
(Pickering, <i>et al.</i> , 2013)	Rats/Wistar/♂	Sub-chronic: 5 days daily injection	2 days	5 mg/kg	Y maze	Deficits in working memory
(Abdul-Monim, <i>et al.</i> , 2007)	Rats/Lister hooded/ ♀	Sub-chronic- 7 days twice daily injections	7 days	5 mg/kg	Operant reversal- learning paradigm	Deficits in executive function
(Jentsch and Taylor, 2001)	Rats/Sprague-Dawley/♂	Sub-chronic- 7 days twice daily injections	7 days	5 mg/kg	Visual Discrimination Testing Conditioned Reinforcement Testing	Deficits in executive function
(Li <i>et al.</i> , 2003)	Rats/Sprague-Dawley/♂ Mice/C57BL/6J/♂	Sub-chronic: 14 days daily injections	7 days	10 mg/kg	RAM	No deficits in working memory

MWM, Morris water maze; NORT, novel-object recognition test; RAM, radial-arm maze.

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