Reassessment of amphetamine- and phencyclidine-induced locomotor hyperactivity as a model of psychosis-like behavior in rats

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Locomotor hyperactivity induced by psychotomimetic drugs, such as amphetamine and phencyclidine, is widely used as an animal model of psychosis-like behaviour and is commonly attributed to an interaction with dopamine release and N-methyl-D-aspartate (NMDA) receptors, respectively. However, what is often not sufficiently taken into account is that the pharmacological profile of these drugs is complex and may involve other neurotransmitter/receptor systems. Therefore, this study aimed to assess the effect of three antagonists targeting different monoamine pathways on amphetamine- and phencyclidine-induced locomotor hyperactivity. A total of 32 rats were pre-treated with antagonists affecting dopaminergic, noradrenergic and serotonergic transmission: haloperidol (0.05 mg/kg), prazosin (2 mg/kg) and ritanserin (1 mg/kg), respectively. After 30 min of spontaneous activity, rats were injected with amphetamine (0.5 mg/kg) or phencyclidine (2.5 mg/kg) and distance travelled, stereotypy and rearing recorded in photocell cages over 90 min. Pre-treatment with haloperidol or prazosin both reduced amphetamine-induced hyperactivity although pre-treatment with ritanserin had only a partial effect. None of the pre-treatments significantly altered the hyperlocomotion effects of phencyclidine. These findings suggest that noradrenergic as well as dopaminergic neurotransmission is critical for amphetamine-induced locomotor hyperactivity. Hyperlocomotion effects of phencyclidine are dependent on other factors, most likely NMDA receptor antagonism. These results help to interpret psychotomimetic drug-induced locomotor hyperactivity as an experimental model of psychosis.

Keywords

Amphetamine; Phencyclidine; Dopamine; Noradrenaline; Serotonin; Psychosis

1. Introduction

Psychosis is a common clinical manifestation of many psychiatric conditions including schizophrenia, bipolar disorder and depression [1]. Amphetamine and phencyclidine are psychotomimetic drugs, extensively used to study the pathophysiology of psychosis as they produce behavioral changes in animals that can model psychosis-like behaviors in humans [2]. The behavioral and biochemical effects of amphetamine and phencyclidine in animals have close parallels in humans. For example, acute administration of amphetamine and phencyclidine results in increased locomotor activity in animals and psychomotor agitation in humans [2, 3]. In many studies, amphetamine-induced hyperactivity is used as a model of dopamine release [2] whereas the action of phencyclidine is usually assumed to be related to N-methyl-D-aspartate (NMDA) receptor antagonism [4, 5]. However, the literature on the pharmacology of these drugs is quite inconsistent reflecting multifaceted complexities associated with psychotomimetic drug molecular actions that remain unresolved.

Some studies conclude that amphetamine-induced locomotor hyperactivity is critically dependent on dopamine release in the nucleus accumbens and can therefore be used to model a psychosis-like hyperdopaminergic state [6, 7]. These studies must be interpreted with caution as other evidence points to a variety of independent mechanisms involved in the effect of amphetamine on dopamine transmission. For example, amphetamine can reduce the release of dopamine, it can block vesicular monoamine transporter activity and activate dopamine D_2 receptor feedback inhibition [8]. Moreover, other studies show that dopamine release itself is not sufficient to elicit all amphetamine-induced behavioral responses. Amphetamine also stimulates noradrenaline and serotonin release from presynaptic terminals [9, 10] and serotonin 5-HT_{2A} receptors regulate the activation of dopaminergic neurons in the nucleus accumbens and striatum [6]. A recent study has shown that noradrenaline release from the reticular nuclei in the brainstem contributes to

amphetamine-induced locomotor hyperactivity and stereotypy [11]. Additionally, noradrenaline and dopamine form a highly interconnected system within the central nervous system and this connectivity allows amphetamine to produce a number of behavioral effects [11].

In contrast to amphetamine, it is generally accepted that the action of phencyclidine on psychosis-like behaviors is due to NMDA receptor hypofunction [4, 5, 12]. Acute phencyclidine administration in rats produces hyperlocomotion that has translational relevance to positive symptoms in humans [12, 13]. However, there is evidence attributing at least part of phencyclidine's action to dopaminergic, noradrenergic and serotonergic transmission [14, 15]. Several studies suggest dopaminergic involvement in hyperlocomotion effects of phencyclidine [16–19] and, according to one of these studies, phencyclidine is able to cause dysregulation in frontal dopamine release [17]. Moreover, serotonergic neurotransmission seems to be critical for the regulation of phencyclidine-induced locomotor hyperactivity [20], and phencyclidine-induced glutamate efflux in frontal cortical regions is modulated by serotonin $5HT_{2A}$ receptors [4, 21]. Thus, it remains unclear how to interpret pharmacological mechanisms involved in the hyperlocomotion effects associated with amphetamine and phencyclidine administration.

The aim of this study was to re-evaluate dopaminergic, noradrenergic and serotonergic involvement in hyperlocomotion induced by amphetamine or phencyclidine, specifically by examining the inhibitory effects of haloperidol, prazosin and ritanserin, which target dopaminergic, noradrenergic and serotonergic activity, respectively [22–24].

2. Materials and methods

2.1 Animals

The experimental protocol was carried out in 32 male Sprague-Dawley rats (Department of Pathology, University of Melbourne). The rats were housed under standard conditions in groups of 2–3, with free access to food and water. They were maintained on a 12 h: 12 h light/dark cycle (lights on at 0700 h) at a constant temperature of 21 ± 2 °C. One week prior to experiments, rats were handled each day over a five-day period. At the time of the first experiment, rats weighed between 250–300 g. All experiments were conducted at the Behavioral Neuroscience Laboratory at the Mental Health Research Institute (Parkville, VIC, Australia). The experimental protocol was approved by the Animal Experimentation Ethics Committee of the University of Melbourne, Australia.

2.2 Drugs and solutions

D-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO, USA; 0.5 mg/kg) and phencyclidine HCl (PCP, Sigma; 2.5 mg/kg) were dissolved in 0.9% saline and injected subcutaneously (s.c.) in the nape of the neck using an injection volume of 1 mL/kg of body weight. Haloperidol (Serenace®, 5 mg ampoules, Searle Laboratories, Crows Nest, NSW, Australia) was diluted to the required doses (0.05 mg/kg) in

saline; vehicle treatment was saline. Prazosin (Sigma) was dissolved in hot water and then diluted to 2 mg/kg in saline, while ritanserin (Sigma) was dissolved in DMSO (1%) and then diluted in saline to give a dose of 1 mg/kg; vehicle treatment consisted of half the rats receiving saline and half receiving 1% DMSO in saline. The three drugs were administered intraperitoneally (i.p.) in an injection volume of 1 mL/kg of body weight. The dose selection for each drug was based on the unpublished and published work completed in our laboratory [25–27] as well as the literature [23, 24].

2.3 Behavioral testing

All experiments were carried out in the morning between 08:30 and 11:30. To minimise the impact of circadian rhythms on drug action, the same time of the light phase i.e., morning was chosen for the study. The study included 4 groups of n = 8 rats/group; 2 groups were treated with amphetamine and 2 groups with phencyclidine. Using a repeated-measures design, these groups were randomly pretreated with either (1) vehicle and haloperidol (0.05 mg/kg), or (2) vehicle, prazosin (2 mg/kg) and ritanserin (1 mg/kg), before being treated with amphetamine or phencyclidine, with 3 days clearance allowed between each pre-treatment. Rats were pre-treated i.p. with either vehicle or drug 15 min before being placed in the photocell cages. After 30 min of spontaneous activity in the photocell cages, rats were injected with either 0.5 mg/kg amphetamine or 2.5 mg/kg phencyclidine and behavioral responses recorded over a further 90 min. Behavioral responses such as distance travelled, stereotypy and vertical counts/rearing were monitored using eight automated photocell cages (43 × 43 × 31 cm, ENV-520, MED Associates, St. Albans, VT, USA) as previously described [20, 26]. Briefly, the position of the rat at any time was detected with sixteen evenly-spaced infrared sources and sensors on each of the four sides of the monitor. These infrared sources/sensors form an array of invisible (virtual) boxes that have a dimension of 4 × 4 sources/sensors (~10.75 × 10.75 cm). Stereotypy, which is considered repetitive behaviours such as circling and head weaving, was automatically determined by the system as small, repetitive beam breaks within a virtual box of 4 × 4 sources/sensors around the rat. The addition of a photobeam array above a rat added a second plane of detection to the system to detect rearing i.e., vertical beam breaks or vertical counts. Every 50 msec, the software checked for the presence or absence of the infrared beam at each sensor, allowing to very precisely track the movement of a rat.

2.4 Data analysis

Data were expressed as the mean ± the standard error of the mean (SEM). All data were analyzed with analysis of variance (ANOVA) with repeated measures where appropriate, using the statistical software package SYSTAT 13.0 (SPSS Inc., Chicago, IL, USA). Data were considered significant at $p < 0.05$. Locomotor activity data were summed in 30 min blocks (baseline, 30 min of pre-treatment only; 0–30

min, 30–60 min and 60–90 min blocks are pre-treatment and treatment) and these four blocks ('Time'; repeated measures) were used to assess the main effect of 'Pre-treatment' (vehicle, haloperidol, prazosin and ritanserin), on amphetamine- or phencyclidine-induced locomotor hyperactivity. The 5–10 min interval during which rats were removed from locomotor monitors to be injected was excluded from data analysis; all other data was included. For significant 'pre-treatment $\times$ time' interactions, post-hoc tests consisted of further pairwise ANOVAs comparing vehicle and pre-treatment for each 30 min block. ANOVA comparing the effects of vehicle, prazosin and ritanserin pre-treatment on amphetamine-induced locomotor hyperactivity revealed a main effect of pre-treatment ($F_{2,14}$ = 12.5, p = 0.001), thus we separated the pre-treatments in the Results. Effect size is estimated with partial eta squared (η^2; IBM SPSS Statistics 26.0, (IBM Corp., Chicago, IL, USA)), where η^2 = 0.01 indicates a small effect, η^2 = 0.06 a medium effect and η^2 = 0.14 a large effect.

3. Results

3.1 Haloperidol - amphetamine

When analyzing the time course of distance travelled, there was a significant pre-treatment $\times$ time interaction ($F_{3,21}$ = 4.9, p = 0.010, η^2 = 0.41) as well as main effects of haloperidol pre-treatment ($F_{1,7}$ = 6.4, p = 0.04, η^2 = 0.48) and of time ($F_{3,21}$ = 34.9, p < 0.001, η^2 = 0.83). These results reflect a significant reduction in amphetamine-induced distance travelled caused by haloperidol (Fig. 1A). When comparing vehicle and 0.05 mg/kg haloperidol at each 30 min time block, haloperidol pre-treatment significantly reduced amphetamine-induced hyperactivity at the 0–30 and 30–60 min time blocks ($F_{1,7}$ = 17.0, p = 0.004, η^2 = 0.71 and $F_{1,7}$ = 6.1, p = 0.042, η^2 = 0.47, respectively) but not at the 60–90 min block (Fig. 1A). Furthermore, haloperidol did not significantly reduce baseline.

Analysis of stereotypic scores supported the distance travelled findings. There was a pre-treatment $\times$ time interaction ($F_{3,21}$ = 3.8, p = 0.04, η^2 = 0.33) as well as a main effect of time ($F_{3,21}$ = 41.7, p < 0.001, η^2 = 0.86), reflecting a haloperidol-induced reduction in amphetamine-induced stereotypy at the 0–30 and 30–60 min time blocks only ($F_{1,7}$ = 5.7, p = 0.05, η^2 = 0.45 and $F_{1,7}$ = 5.3, p = 0.05, η^2 = 0.43, respectively; Fig. 2A). Similarly, when analyzing vertical scores, there was a significant pre-treatment $\times$ time interaction ($F_{3,21}$ = 4.8, p = 0.01, η^2 = 0.41) as well as main effects of pre-treatment ($F_{1,7}$ = 5.0, p = 0.06, η^2 = 0.42) and of time ($F_{3,21}$ = 50.9, p < 0.001, η^2 = 0.88). The significant interaction was due to a haloperidol-induced reduction in amphetamine-induced rearing at the 0–30 and 30–60 min time blocks only ($F_{1,7}$ = 6.8, p = 0.04, η^2 = 0.49 and $F_{1,7}$ = 6.0, p = 0.04, η^2 = 0.46, respectively; Table 1).

3.2 Haloperidol - phencyclidine

Analysis of the time course of distance travelled revealed a main effect of time ($F_{3,21}$ = 27.6, p < 0.001, η^2 = 0.80) reflecting an increase in distance travelled after phencycli-

Table 1. Cumulative vertical counts after treatment with amphetamine and phencyclidine.

	Amphetamine	Phencyclidine
Vehicle	2060 ± 206	710 ± 170
Haloperidol	1452 ± 118*	503 ± 108
Vehicle	1730 ± 290	726 ± 119
Prazosin	697 ± 233*	575 ± 132
Ritanserin	1702 ± 264	957 ± 231*

Rats were pre-treated with vehicle, haloperidol (0.05 mg/kg), prazosin (2 mg/kg) or ritanserin (1 mg/kg) and vertical counts/rearing were obtained during 90 min after injection of amphetamine (0.5 mg/kg) or phencyclidine (2.5 mg/kg). Differences between pre-treatments were analyzed by ANOVA. Data are expressed as total counts (group average) ± SEM. *p < 0.05 compared to vehicle pre-treatment.

dine. There was no significant main effect of haloperidol pre-treatment or pre-treatment $\times$ time interaction, reflecting a lack of effect of 0.05 mg/kg haloperidol on phencyclidine-induced hyperactivity (Fig. 1B), despite this dose being effective against amphetamine-induced hyperactivity. Similarly, when analyzing stereotypic and vertical scores, there was a main effect of time ($F_{3,21}$ = 38.3, p < 0.001, η^2 = 0.85 and $F_{3,21}$ = 6.5, p = 0.003, η^2 = 0.48, respectively), but no other significant effects, again confirming the lack of effect of haloperidol on phencyclidine-induced hyperactivity (Fig. 2B; Table 1).

3.3 Prazosin - amphetamine

Analysis of distance travelled showed a significant pre-treatment $\times$ time interaction ($F_{3,21}$ = 7.2, p = 0.002, η^2 = 0.51) and main effects of prazosin pre-treatment ($F_{1,7}$ = 13.5, p = 0.008, η^2 = 0.66) and time ($F_{3,21}$ = 16.0, p < 0.001, η^2 = 0.70). When comparing vehicle and prazosin at each 30 min time block, they all showed a significant prazosin-induced reduction in distance travelled (baseline: $F_{1,7}$ = 12.5, p = 0.01, η^2 = 0.64; 0–30 min: $F_{1,7}$ = 12.2, p = 0.01, η^2 = 0.64; 30–60 min: $F_{1,7}$ = 10.4, p = 0.015, η^2 = 0.60; 60–90 min: $F_{1,7}$ = 15.6, p = 0.006, η^2 = 0.69) (Fig. 1C).

There were significant main effects of prazosin pre-treatment ($F_{1,7}$ = 8.8, p = 0.021, η^2 = 0.56) and time ($F_{3,21}$ = 15.9, p < 0.001, η^2 = 0.69) on stereotypy scores, but no pre-treatment $\times$ time interaction, suggesting that prazosin pre-treatment reduced stereotypy regardless of the presence of amphetamine (Fig. 2C). In regards to vertical scores, there was a significant pre-treatment $\times$ time interaction ($F_{3,21}$ = 8.3, p = 0.001, η^2 = 0.54) and main effects of prazosin pre-treatment ($F_{1,7}$ = 18.9, p = 0.003, η^2 = 0.73) and time ($F_{3,21}$ = 14.5, p < 0.001, η^2 = 0.68). When comparing vehicle and prazosin at each time block, prazosin pre-treatment did not alter baseline rearing, but did reduce amphetamine-induced rearing (0–30 min: $F_{1,7}$ = 21.5, p = 0.002, η^2 = 0.75; 30–60 min: $F_{1,7}$ = 12.4, p = 0.01, η^2 = 0.64; 60–90 min: $F_{1,7}$ = 23.6, p = 0.002, η^2 = 0.77).
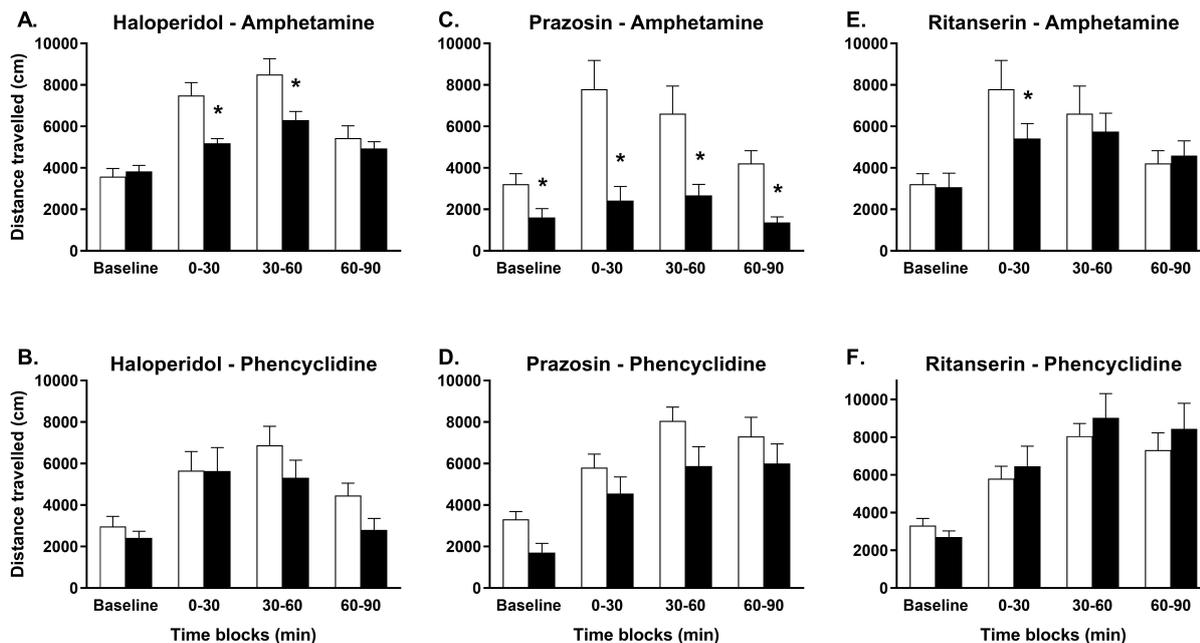
Fig. 1. Distance travelled after amphetamine (top panels: A, C, E) or phencyclidine (bottom panels: B, D, F) in rats pre-treated with vehicle (white bars) or psychotomimetic-drug (black bars; A, B: haloperidol 0.05 mg/kg, C, D: prazosin 2 mg/kg, E, F: ritanserin 1 mg/kg). Spontaneous activity ('Baseline') was recorded for 30 min before rats were injected with amphetamine (0.5 mg/kg) or phencyclidine (2.5 mg/kg) and activity recorded for a further 90 min (time blocks: 0–30 min, 30–60 min, 60–90 min). Bars represent total distance travelled in 30 min (group average; cm) $\pm$ SEM; *$p < 0.05$ compared to vehicle pre-treatment of that time block (this analysis was only done if there was a significant pre-treatment $\times$ time interaction).

3.4 Prazosin - phencyclidine

There were significant main effects of prazosin pre-treatment ($F_{1,7}$ = 7.1, p = 0.032, η^2 = 0.50) and time ($F_{3,21}$ = 45.9, $p < 0.001$, η^2 = 0.87) on distance travelled, but no pre-treatment $\times$ time interaction. This suggests that the reduction in distance travelled caused by 2 mg/kg prazosin occurred similarly with or without phencyclidine treatment (Fig. 1D).

Analysis of stereotypy scores showed a significant pre-treatment $\times$ time interaction ($F_{3,21}$ = 7.6, p = 0.001, η^2 = 0.52), a trend for a main effect of prazosin pre-treatment ($F_{1,7}$ = 5.6, p = 0.05, η^2 = 0.44), and a significant main effect of time ($F_{3,21}$ = 47.5, $p < 0.001$, η^2 = 0.87). Further analysis of the interaction revealed a significant effect of prazosin pre-treatment on baseline ($F_{1,7}$ = 19.4, p = 0.003, η^2 = 0.74) but no effect on the phencyclidine-induced increase in stereotypy scores (Fig. 2D). When analyzing vertical scores, there was a significant main effect of time ($F_{3,21}$ = 18.1, $p < 0.001$, η^2 = 0.72), but no other significant effects, suggesting that prazosin pre-treatment had no effect on rearing (Table 1).

3.5 Ritanserin - amphetamine

When analyzing distance travelled, there was a significant pre-treatment $\times$ time interaction ($F_{(3,21)}$ = 4.5, p = 0.014, η^2 = 0.39), a main effect of time ($F_{3,21}$ = 17.7, $p < 0.001$, η^2 = 0.72), but no significant main effect of ritanserin pre-treatment (Fig. 1E). Comparing vehicle and 1 mg/kg ritanserin at each time block revealed that ritanserin pre-treatment did not alter baseline, but significantly reduced amphetamine-induced hyperactivity at the 0–30 min time block only ($F_{1,7}$ = 7.6, p = 0.028, η^2 = 0.52).

Analysis of stereotypy scores revealed a main effect of time ($F_{3,21}$ = 21.2, $p < 0.001$, η^2 = 0.75), but no significant main effect of ritanserin pre-treatment or a time $\times$ pre-treatment interaction suggesting that ritanserin did not affect stereotypy (Fig. 2D). When analyzing vertical scores, there was a main effect of time ($F_{3,21}$ = 30.7, $p < 0.001$, η^2 = 0.81), and a weak pre-treatment $\times$ time interaction ($F_{3,21}$ = 3.1, p = 0.047, η^2 = 0.31), but no significant main effect of ritanserin pre-treatment (Table 1). Further analysis of the interaction revealed a trend for a reduction in amphetamine-induced rearing occurred at the 0–30 min time block ($F_{1,7}$ = 5.0, p = 0.06, η^2 = 0.42).

3.6 Ritanserin - phencyclidine

Analysis of distance travelled revealed a main effect of time ($F_{3,21}$ = 27.5, $p < 0.001$, η^2 = 0.80) but no other significant effects, suggesting that 1 mg/kg ritanserin had no effect on baseline or phencyclidine-induced distance travelled (Fig. 1F).

Analysis of stereotypy scores revealed a pre-treatment $\times$ time interaction ($F_{3,21}$ = 3.9, p = 0.024, η^2 = 0.36) and a main effect of time ($F_{3,21}$ = 32.1, $p < 0.001$, η^2 = 0.82), but no significant main effect of ritanserin pre-treatment (Fig. 2F).
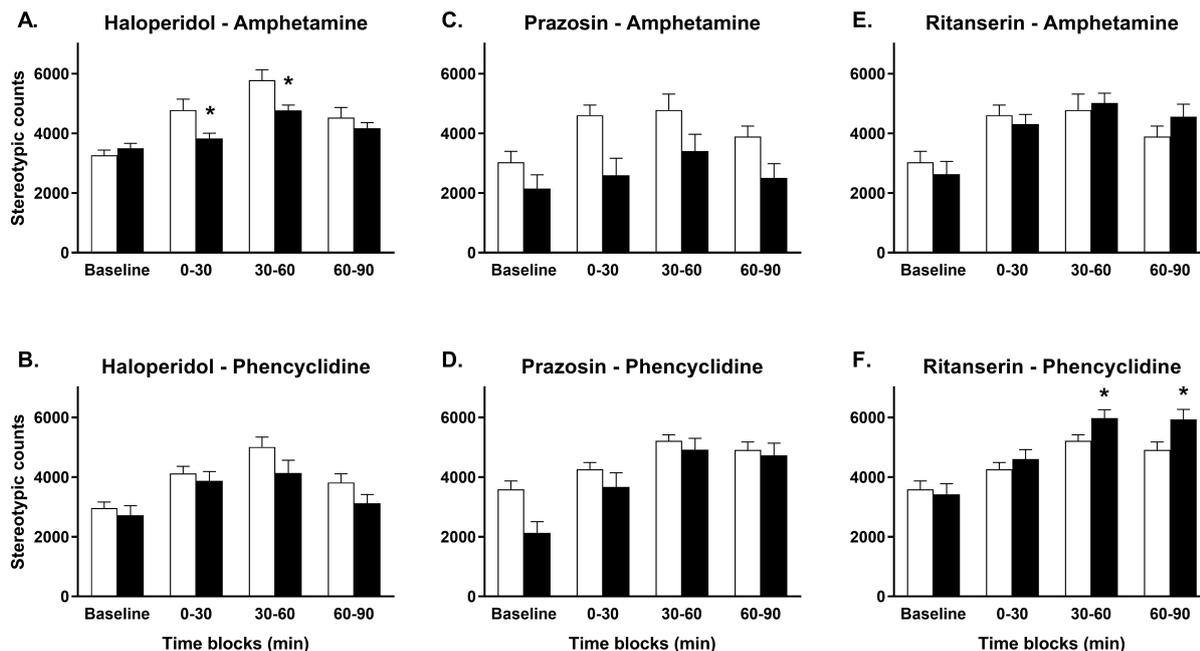
Fig. 2. Stereotypic counts after amphetamine (top panels: A, C, E) or phencyclidine (bottom panels: B, D, F) in rats pre-treated with vehicle (white bars) or psychotomimetic-drug (black bars; A, B: haloperidol 0.05 mg/kg, C, D: prazosin 2 mg/kg, E, F: ritanserin 1 mg/kg). Spontaneous activity ('Baseline') was recorded for 30 min before rats were injected with amphetamine (0.5 mg/kg) or phencyclidine (2.5 mg/kg) and activity recorded for a further 90 min (time blocks: 0–30 min, 30–60 min, 60–90 min). Bars represent total stereotypic counts in 30 min (group average) $\pm$ SEM; *$p < 0.05$ compared to vehicle pre-treatment of that time block (this analysis was only done if there was a significant pre-treatment $\times$ time interaction).

When comparing vehicle and ritanserin at each time block, ritanserin pre-treatment did not alter baseline stereotypy, but did enhance phencyclidine-induced stereotypy (30–60 min: $F_{1,7} = 7.0$, $p = 0.033$, $\eta^2 = 0.50$; 60–90 min: $F_{1,7} = 12.0$, $p = 0.011$, $\eta^2 = 0.63$). Similarly, when analyzing vertical scores, there was a pre-treatment $\times$ time interaction ($F_{3,21} = 3.3$, $p = 0.04$, $\eta^2 = 0.32$) and a main effect of time ($F_{3,21} = 11.7$, $p < 0.001$, $\eta^2 = 0.63$; Table 1). Further analysis of the interaction revealed there was only a trend for ritanserin to enhance phencyclidine-induced rearing at the 60–90 min time block ($F_{1,7} = 4.0$, $p = 0.087$, $\eta^2 = 0.36$).

4. Discussion

In this study, we used three different antagonists to evaluate their inhibitory effects on amphetamine- and phencyclidine-induced locomotor hyperactivity. The principal findings of this study were that: (1) amphetamine-induced locomotor hyperactivity was attenuated by pre-treatment with haloperidol and prazosin; (2) amphetamine-induced locomotor hyperactivity was partially reduced by ritanserin; (3) phencyclidine-induced locomotor hyperactivity was not affected by either antagonist. These findings suggest that dopaminergic as well as noradrenergic neurotransmission is critical for the regulation of hyperlocomotion effects of amphetamine while phencyclidine-induced hyperactivity is dependent on other factors, most likely NMDA receptor

antagonism or other not yet known mechanisms but, importantly, not dopaminergic or noradrenergic mechanisms.

Psychotomimetic drug-induced locomotor hyperactivity is generally attributed to limbic-striatal modulation of brainstem motor circuits [2]. Classic micro-injection and lesion studies have highlighted the role of dopamine in the nucleus accumbens (ventral striatum) in modulating the ambulatory locomotor response to amphetamine, while dopamine in the caudate putamen (dorsal striatum) is instead involved in the stereotypy/rearing induced by amphetamine [28–31]. The nucleus accumbens is an important regulatory interface between limbic and motor systems in driving adaptive behavior through inputs from the prefrontal cortex, hippocampus and amygdala, and outputs to the ventral pallidum and substantia nigra [32]. Recent advances in human neuroimaging techniques call into question the involvement of the mesolimbic system in relation to psychotic symptoms and instead point to the importance of dopaminergic nigrostriatal pathways, specifically in the dorsal striatum [33, 34].

Haloperidol, the predominantly dopamine D_2 receptor antagonist, and prazosin, an adrenergic α_1 receptor antagonist, attenuated amphetamine-induced locomotor hyperactivity, including distance travelled, stereotypy and rearing. While the present study found that prazosin reduced baseline activity, suggesting that some of its effect on amphetamine may be due to non-specific effects, importantly, prazosin also

significantly attenuated amphetamine-induced ambulatory locomotion. This is consistent with literature showing that haloperidol reverses hyperlocomotion [35, 36] and prazosin attenuates locomotor hyperactivity induced by amphetamine [37] or methamphetamine [38, 39]. In terms of rearing, it was expected that haloperidol pre-treatment would reduce amphetamine-induced rearing given haloperidol's mechanism of action and the role of dopamine in mediating hyperlocomotion/rearing. The attenuation of amphetamine-induced hyperlocomotion/rearing by prazosin may be attributed to the activation of postsynaptic adrenergic α_1 receptors and the strong interplay between noradrenaline and dopamine in amphetamine-induced behaviors [11, 38]. For example, noradrenergic axons from the locus coeruleus regulate dopamine release throughout the brain, including the ventral striatum and dorsal striatum [11]. The (partial) effect of ritanserin on amphetamine-induced locomotor hyperactivity and no effect on rearing is in line with earlier studies showing that 5-HT$_{2A}$ receptors modulate dopamine release in the nucleus accumbens and ventral striatum [6] and that a specific serotonin 5HT$_{2A}$ receptor antagonist (SR46349B) inhibited amphetamine-induced hyperactivity in rats [38, 39].

Our findings suggest a complex overlap of dopaminergic D$_2$ and adrenergic α_1 receptors in the brain in the hyperlocomotion effects of amphetamine. High levels of dopamine D$_2$ receptors are found in the striatum, nucleus accumbens and olfactory tubercle [40], whereas high levels of adrenergic α_1 receptors are located in hypothalamic nuclei, substantia nigra, but also in the nucleus accumbens [41]. It was shown that dopamine release is controlled by noradrenaline stimulation of α_1 adrenergic receptors in the prefrontal cortex [42] and that stimulation of postsynaptic adrenergic α_{1b} receptors increases dopamine-mediated locomotor responses [43]. Thus, activation of α_{1b} adrenoceptors by noradrenaline in the frontal cortex modulates dopamine release in the nucleus accumbens and thus hyperlocomotion effects of amphetamine. The effect of prazosin in the present study could be explained by an action on this frontal cortical pathway. However, an alternative, or additional mechanism by which α_1 receptors may be involved in amphetamine locomotor hyperactivity is by a more direct interaction in the nucleus accumbens [37, 44, 45]. Both α_1 receptors located presynaptically on noradrenergic terminals in the nucleus accumbens as well as α_1 receptors located postsynaptically from these terminals, are involved in the regulation of dopamine release [46]. Blockade of the receptors by prazosin could then inhibit evoked dopamine release [46] and, hence, the effect of amphetamine [45].

The effect of ritanserin via 5-HT$_{2A}$ receptor antagonism is most likely located in the basal ganglia where these receptors are found in high levels [47]. It cannot be excluded that the lack of complete inhibition by ritanserin could be due to the dose being too low to occupy adequate level of 5HT$_{2A/2C}$ receptors and/or a possibility of 'wearing off' effect (signifi-cantly inhibited amphetamine's effects during the 0–30 min time block only). Future studies should examine different doses of acutely administered ritanserin on amphetamine-induced hyperlocomotion as well as the kinetics of ritanserin including its metabolism and elimination, to establish the role of serotonergic transmission in amphetamine-induced hyperlocomotion.

In contrast to amphetamine, phencyclidine-induced hyperactivity was not affected by any of the three antagonists used. Given that dopamine D$_2$, adrenergic α_1, or serotonin 5-HT$_{2A/2C}$ receptor antagonism did not markedly alter phencyclidine-induced hyperactivity, the role of glutamate NMDA receptors in phencyclidine's action remains the dominating hypothesis for now. However, there may be other mechanisms not yet known involved. Hence, to rule out or confirm selective NMDA receptor involvement, future studies must consider including glutamate NMDA receptor agonists/antagonists in the pharmacological profiling of phencyclidine-induced locomotor hyperactivity. There was, however, a slight effect for ritanserin to enhance phencyclidine-induced stereotypy and rearing. This finding is in line with the above-mentioned notion that other mechanisms including serotonin-glutamate interactions may be involved [26]. Given that stereotypy/rearing, but not distance travelled, were affected by ritanserin, it is possible that the dorsal striatum rather than the ventral striatum is involved, however further studies are required. Moreover, this highlights that distance travelled, stereotypy and rearing may all have different brain regions and circuitry governing them and, consequently, different pharmacology.

There are several limitations of this study. Firstly, only male rats were used in this study. Given that we and others have shown sex differences in psychotomimetic-induced behaviors in animals [48, 49], future studies should include both male and female rats. Second, future studies should consider circadian rhythms and whether testing should occur during the light or dark phase. However, doses of amphetamine (0.6 mg/kg) similar to that used in this study were found to increase locomotor activity regardless of the large difference in baseline activity between the light and dark phases [50]. Third, the locomotor photocell system automatically measured 'stereotypy' as any repetitive beam breaks within a virtual box, however the exact behaviour within the virtual box, such as circling and/or head weaving, was not well-defined. Finally, we have not directly examined NMDA receptor antagonism in phencyclidine-induced behaviours, hence, at this stage we can only assume that NMDA receptor hypoactivity is involved. Future research should use selective NMDA receptor agonists and antagonists to dissociate the role of glutamatergic NMDA receptors in phencyclidine-induced locomotor hyperactivity.

5. Conclusions

This study shows that the noradrenergic, as well as the dopaminergic system, is involved in mediating

amphetamine- but not phencyclidine-induced locomotor hyperactivity. In addition to dopamine D_2 and adrenergic α_1 receptors, our study showed that serotonin 5-$HT_{2A/2C}$ receptors also play a small role in amphetamine-induced hyperactivity. By contrast, hyperlocomotion induced by phencyclidine is likely dependent on NMDA receptors. Future studies should explore the role of other neurotransmitter systems, such as the cholinergic system [11], in mediating the locomotor effects induced by amphetamine and phencyclidine. These findings highlight the complex pharmacology involved in the commonly-used psychosis model of psychotomimetic drug-induced locomotor hyperactivity, and suggest caution is warranted in the interpretation of the neurotransmitter-receptor systems involved.

Abbreviations

ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; HCl, hydrochloric acid; NMDA, N-methyl-D-aspartate.

Author contributions

SK and MvdB designed the research study. SK performed the research. AG analyzed the data. SK and AG wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animals were raised and handled at the Department of Pathology, the University of Melbourne, Melbourne, Australia until 5 weeks of age. They were then transferred to the Behavioral Neuroscience Laboratory at the Mental Health Research Institute in Melbourne (Parkville, VIC Australia) where all experiments were conducted. The experimental protocol was approved by the Animal Experimentation Ethics Committee of the University of Melbourne, Melbourne, Australia (AEEC #01159). All scientific procedures using animals were carried out in accordance with *the Prevention of Cruelty to Animals Act 1986* and the *Australian code of practice for the care and use of animals for scientific purposes* (1997).

Conflict of interest

The authors declare no conflict of interest.

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