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Environmental enrichment ameliorates phencyclidine-induced cognitive deficits

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ABSTRACT

Recent work has suggested that environmental enrichment during development can enhance aspects of learning and memory, however its effects on executive function and cognitive flexibility have not been well studied. The goal of this research was to evaluate whether environmental enrichment (EE) that included wheel running would improve cognitive performance in young male Long Evans rats that received subchronic administration of either phencyclidine (PCP) or saline. We used a sensitive extradimensional/intradimensional (ED/ID) test of cognitive flexibility similar to that used in humans and nonhuman primates for assessing executive function. PCP-treated rats demonstrated a selective impairment on ED shift (EDS) performance without significant impairment on other discrimination problems when compared to saline treated control animals. A separate group of animals that received PCP + EE demonstrated significantly improved performance on EDS and reversal learning problems, whereas the saline + EE group demonstrated a non-selective improvement in overall performance when compared to non-enriched saline controls. The saline + EE group demonstrated greater activity levels as measured by wheel running when compared to the PCP + EE group, but no significant associations were found between wheel running and cognitive performance. Together, these data suggest that EE that features wheel running may have promoted a general cognitive enhancement while also selectively acting upon neurobiological mechanisms that subserve executive function and cognitive flexibility in impaired animals. Development of novel treatment methodologies that target mechanisms underlying the ameliorative effects of EE in this model of cognitive impairment may be a useful tool in the development of new therapeutic strategies for disorders that feature cognitive dysfunction as a key symptom.

1. Introduction

Cognitive deficits found in patients with schizophrenia appear widespread and are related to executive function, working memory, and attention (Harvey, 2010). Moreover, cognitive impairments associated with schizophrenia are not effectively treated by current medications and recently greater resources have been directed toward developing better preclinical models of these neuropsychiatric disorders (Nestler and Hyman, 2010). Traditional and atypical antipsychotics can often effectively treat the positive and negative symptoms of schizophrenia but cognitive dysfunction continues to be the most functionally debilitating aspect of schizophrenia (Green, 1996). Specifically, higher degrees of impairment are associated with greater difficulty reintegrating into society and poorer outcome (Harvey, 2010).

A large line of evidence indicates that cognitive impairment appears to be a good predictor of earlier disease onset (Gold, 2004). Several studies have reported that cognitive impairment can manifest itself in early childhood long before positive symptoms onset, compromising attentional abilities and academic success (Niendam et al., 2003). Early onset of these cognitive symptoms makes them an attractive target for intervention as a potential means of preventing or delaying emergence of schizophrenia (Harvey, 2009). Drug development has been guided by the knowledge of potentially dysregulated neurotransmitter systems underlying the cognitive impairment seen in schizophrenia. The use of animal models to model key symptoms of neuropsychiatric disorders has allowed examination into potential underlying neurobiological mechanisms.

One experimental method employed to model cognitive deficits has been the subchronic suppression of N-methyl-D-aspartate (NMDA) receptor activity (Rodefer et al., 2005; Goetghebeur and Dias, 2009). Animal models investigating NMDA receptor (NMDAR) dysfunction have been prevalent in recent literature (cf. Castner et al. 2004) and produce cognitive impairments similar to those seen in schizophrenia (Sircar, 2003). Recent work from our lab (Rodefer et al., 2005, 2008) and others (Jentsch and Roth, 1999; Egerton et al., 2005; Goetghebeur and Dias, 2009) has demonstrated that subchronic administration with the NMDA receptor antagonist phencyclidine (PCP) administration reliably produces enduring selective deficits in cognitive flexibility. As there are no currently effective pharmacotherapies approved for the treatment of cognitive impairment associated with schizophrenia, this model offers the opportunity to examine potential pro-cognitive treatments that act through this system.

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Unfortunately, the development of novel and effective treatment strategies has been complicated by the complex pathophysiology of schizophrenia and the interactions between genetic and environmental modulators of pathogenesis (Karl et al., 2007; Laviola et al., 2008). Evidence from both preclinical (Weiss and Feldon, 2001; Toua et al., 2010) and clinical (Gallagher et al., 2007; Krabbendam and van Os, 2005) studies have suggested that factors such as isolation rearing or low SES during early development may play a role in vulnerability for the development of schizophrenia like symptoms. There are few clinical studies, but one recent report demonstrated that two years of a daily enrichment program focusing on nutrition, education, and physical exercise between ages 3 and 5 produced lower scores for schizotypal personality and antisocial behavior during early adulthood compared to a matched, non-enriched group (Raine et al., 2003). Indeed, the effect of aerobic activity alone on cognitive enhancement has been reported to improve age-related memory impairment (Hopkins and Bucci, 2010).

Thus, we aimed to combine environmental enrichment manipulations with subchronic PCP administration that model cognitive disruption associated with schizophrenia. In the current experiment, we examined the hypothesis that environmental enrichment would ameliorate a selective PCP-induced impairment in cognitive flexibility. Animals received daily enrichment sessions for a total of one month, with subchronic PCP or saline vehicle treatment occurring mid-way through the enrichment period that corresponds to late adolescence or early adulthood. Cognitive performance of subjects was evaluated on a rodent model of an attentional set-shifting ED/ID task (Birrell and Brown, 2000) that we and others have demonstrated to be sensitive to the effects of natural aging (Barense et al., 2002 Rodefer and Nguyen, 2008), cortical lesions (Fox et al., 2003; Ng et al., 2007) and pharmacological manipulation (Chen et al., 2004, Rodefer et al., 2005, 2008; Goetghebeur and Dias, 2009).

2. Materials and methods

2.1. Subjects

Thirty-two male Long Evans rats (Harlan) arrived in the laboratory at approximately 42–45 days of age and were housed individually in standard polycarbonate cages. Rats were assigned to one of four experimental groups (n = 8 each): saline (subchronic saline treatment + no environmental enrichment), saline + EE (subchronic saline treatment + environmental enrichment), PCP (subchronic PCP treatment + no environmental enrichment) and PCP + EE (subchronic PCP treatment + environmental enrichment). Environmental enrichment sessions and behavioral testing were conducted at a consistent time during the light phase of a 12-hour light/dark cycle (lights on at 7:00 A.M.). Water in home cages and environmental enrichment chambers was always available *ad libitum*. From time of arrival until 7–10 days prior to behavioral testing, food was also available *ad libitum*. During behavioral training and testing, post-session daily food allotments were adjusted to maintain animals at 85–90% of their free feeding weights, ensuring animals were motivated to complete the food-reinforced behavioral task. This also permitted animals to complete the 30 days of environmental enrichment without changes in diet or food availability. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were performed in accordance with the guidelines of the NIH Guide for Care and Use of Laboratory Animals.

2.2. Apparatus

The ED/ID task required rodents to forage for a small food reward (one half piece, Honey-Nut Cheerio; General Mills) that was buried in a pot. This experiment used terracotta flower pots (10 cm H & ID) that were filled with various digging materials and scented with perfumed oils applied to the rim that represented the dimensions of media and odor, respectively. The behavioral testing apparatus was a bottomless and topless box (Plexiglas and polyvinyl chloride construction; approximately 50 cm L × 38 cm W × 25 cm H) with an opaque divider used to separate one-third of the box along the length of the chamber. On each trial, two digging pots were placed adjacent to one another in the larger portion of the chamber while the rat remained in the smaller start box portion in the rear of the box. At the beginning of each trial, the divider was raised to give the rat access to the pots, and subsequently lowered after the rat had fully entered this part of the box to prevent re-entry into the start box.

The four behavioral chambers used for environmental enrichment were standard polycarbonate cages with an attached running wheel (Model EV-046, Med Associates, St. Albans, VT) that displayed wheel revolution counts. The running wheel (36 cm diameter; 0.5 cm stainless steel bars) was securely attached to the polycarbonate enrichment chamber (approximately 48 cm L × 27 cm H × 20 cm W) and rats in EE groups had free access to both the running wheel and enrichment chamber during daily sessions. Small animal and rodent toys (10 different items) that varied in shape, size, color, and texture were purchased from local commercial sources and placed within the chamber. Multiple sets of enrichment items were purchased and allowed the rotation items across chambers in a counterbalanced fashion to control for any differences (e.g., color) across objects. Enrichment chambers were cleaned after each daily session and wiped down with alcohol before filling with clean wood chip bedding. Enrichment items were also cleaned after daily sessions and were repositioned daily to promote novelty and complexity within the enrichment chamber. Non-enriched animals did not have access to the running wheels and enrichment objects.

2.3. Environmental enrichment

In the enrichment conditions (PCP + EE, saline + EE), each rat was placed in an enrichment chamber for daily 1-hour sessions for 30 consecutive days (PD 51–80). Animals had free access to exercise wheels during the daily EE session. Diet enrichment administered in the chambers consisted of a 3-day alternation between tap water (alone), a saccharin solution (0.1% wt/vol), and one-half of a Chips Ahoy cookie (Nabisco, Hanover, NJ). Regular tap water was made concurrently available on days when the saccharin solution and cookie were available. Tap water and the saccharin solution were made available in 500 mL glass bottles placed in the wire lid covering the enrichment chamber. Non-EE animals were maintained solely with standard rat chow (Purina) and tap water.

2.4. Behavioral testing

To accustom rats to the food reward as well as to retrieve food rewards from the pots, we put a pot filled with standard housing bedding and baited with several Cheerios into each rat's home cage for a number of days. After this period, rats were habituated to the testing chambers for 30-minute daily sessions and given access to two pots (with the same standard home cage bedding material), which were continuously re-baited with Cheerios. Rats were given access to these pots for several days until they were reliably and consistently digging to retrieve the food reward. To count as a "dig" the rat was required to displace a significant amount of medium within the pot to retrieve the food reward buried (>2.5 cm) below the surface. As such, the rats could investigate the surface of the digging media in each pot with paws or snout before executing a dig response and these behaviors were not scored as a dig unless significant media had been displaced. Thus, because the rats were allowed to investigate the pot in this manner, it is possible that choices may have been based on other (e.g., tactile or visual) characteristics of the stimuli. A small amount of powdered food reward was added to each pot to ensure that animals could not solve the discrimination problem by olfactory cues. A criterion of six consecutive trials solved correctly was used for all discrimination problems.

Once habituated, all rats were trained on two simple discrimination (SD) learning problems to acquaint the rats with the discrimination learning procedure and the two dimensions of discrimination stimuli (odor and media). SD learning problems challenged the rat to discriminate between two stimuli of the *same* dimension; therefore, during a discrimination problem featuring odor stimuli, the digging medium was held constant, and vice versa. In one complete training session, all rats performed both discrimination problems—an odor discrimination, pairing "exotic" vs. "sensual" (Body Shop perfume oils, Wake Forest, NC) and a digging medium discrimination of pine shavings vs. shredded cardboard. The positive stimuli (S+) associated with the food reward were counterbalanced across all rats and not used again after completion of this training phase. Rats received two SD sessions prior to behavioral testing.

During behavioral testing, a trial began by raising the opaque divider to allow the rat access to the two pots placed in the front two-thirds of the testing chamber—one of which was baited with the food reward. The rat was then able to dig in the pots to find the reward. An error was recorded if the rat first executed a dug response in the incorrect (S−; un-baited) pot. The first four trials of all discrimination problems represented a discovery period in which the rat was allowed to dig in both pots until the food reward was located in an attempt to minimize the potential for premature extinction. Errors were still recorded if the rat initially executed an incorrect dig response, but the rat was allowed to continue digging until the food reward was located. After the 4-trial discovery period, an incorrect response warranted termination of that trial and an immediate return to the start box. Testing on each discrimination problem continued until the rat reached criterion (6 consecutive correct responses).

Each rat progressed through the ED/ID test, which was composed of seven (7) different discrimination problems (see Table 1) within one experimental session (approximately 45–90 min in duration). Initially, rats were presented with a simple discrimination (SD), which required the rat to successfully discriminate between either two odors or two different digging media (as an example: aspen shavings vs. shredded folders). After reaching criterion, subjects moved onto a compound discrimination (CD) with the same S+ (e.g., aspen) as in the SD. However, the CD introduced a new dimension (e.g., odor, if the SD was media, and vice versa) but held constant the correct and incorrect exemplar conditions, such that the new dimension (e.g. odor) was not a reliable predictor of the correct location of the food reward. After CD completion, the CD problem was then reversed (Rev1), such that the previously reinforced stimulus exemplar (S+: aspen) became the unreinforced (S−) stimulus, and vice-versa (e.g., S+ = shredded folders), but with the irrelevant dimension remaining the same and having no predictive validity. After Rev1, new exemplars of each dimension were introduced in an intradimensional shift (IDS) problem where the initial correct dimension (e.g., digging media) remained constant, while new exemplars of odors and media were introduced (e.g. foam rubber and plastic beads as media, patchouli and mulberry as odors). Next, the IDS was reversed (Rev2), just as before in Rev1. Following Rev2, the extradimensional shift (EDS) was introduced, where the previously relevant dimension (e.g., digging media) became the irrelevant dimension, and the previously irrelevant dimension (e.g., odor) became predictive of food reward location. In addition, new exemplars of specific stimuli for the correct and incorrect dimensions were introduced (e.g., gravel and glass beads as media, cinnamon and gardenia as odors). Finally, the EDS problem was reversed (Rev3) so that the previous S+ was now the S−, and vice-versa. The order of discrimination problems remained the same for all animals, but the direction of EDS shift (i.e. medium to odor or odor to medium) was counterbalanced across all subjects within each group ($n=4$ odor; $n=4$ media) and was without effect on any of the behavioral variables ($p>0.05$) and thus was not considered in the presentation of results. The specific order of stimuli was also varied across animals, but an excess of possible pairings did not allow for complete counterbalancing, so pairs of stimuli were assigned in pairs and held constant across all subjects (e.g., when jasmine was the S+, vanilla was the S−) (see Table 1). The order of presentation of the stimuli was equally represented to the greatest degree possible.

2.5. Drug

Phencyclidine HCl (PCP) was purchased from Sigma-Aldrich (St. Louis, MO) and prepared in sterile physiological (0.9% wt/vol) saline. PCP was prepared at a concentration of 5.0 mg/ml and both PCP and saline (vehicle) were administered in a volume of 1 ml/kg (ip). Dose of PCP was selected based on prior work (Rodefer et al., 2005, 2008) and is consistent with work from other laboratories (Egerton et al., 2005). After habituating to the colony room environment, all rats received subchronic injections of either PCP or saline twice daily (approximately 8:00 a.m. and 6:00 p.m.) for a period of 7 days. After the full dosing

Table 1
Example of a possible combination of stimulus pairs for a rat shifting from digging medium to odor as the relevant dimension.

	Dimensions		Exemplar combinations (example)	
Discrimination	Relevant	Irrelevant	S+	S−
Simple discrimination (SD)	**Medium**		**Aspen shavings** vs. shredded folders (no odor)	
Compound discrimination (CD)	**Medium**	Odor	**Aspen shavings**/jasmine vs. shredded folders/vanilla *or* **aspen shavings**/vanilla vs. shredded folders/jasmine	
Reversal1 (Rev1)	**Medium**	Odor	**Shredded folders**/jasmine vs. aspen shavings/vanilla *or* **shredded folders**/vanilla vs. aspen shavings/jasmine	
Intra-dimensional shift (IDS)	**Medium**	Odor	**Foam rubber**/mulberry vs. plastic beads/patchouli *or* **foam rubber**/patchouli vs. plastic beads/mulberry	
Reversal2 (Rev2)	**Medium**	Odor	**Plastic beads**/mulberry vs. foam rubber/patchouli *or* **plastic beads**/patchouli vs. foam rubber/mulberry	
Extra-dimensional shift (EDS)	**Odor**	Medium	Aquarium gravel/**cinnamon** vs. glass beads/gardenia *or* glass beads/**cinnamon** vs. aquarium gravel/gardenia	
Reversal3 (Rev3)	**Odor**	Medium	Aquarium gravel/**gardenia** vs. glass beads/cinnamon *or* glass beads/**gardenia** vs. aquarium gravel/cinnamon	

Note: approximately half of the rats switched from medium to odor, and half switched from odor to medium. The correct exemplar is shown in bold, and can be paired with either exemplar from the irrelevant dimension across trials within each discrimination problem. In the IDS and EDS, the stimuli were novel exemplars of each dimension.

period, rats experienced a washout period of 7 days before behavioral training and testing. All PCP treated animals received the drug regimen at the same developmental time period (PD 66–72, which corresponded to days 15–21 of the 30 day environmental enrichment period).

2.6. Data analysis

Given previous findings that EDS problems are more difficult to solve compared to IDS problems (Barense et al., 2002; Fox et al., 2003; Rodefer et al., 2005, 2008), we first assessed the validity of the cognitive set in saline-treated control animals using a paired *t* test. We then examined the validity of the selective nature of the PCP-induced EDS deficits that has been reported previously (Rodefer et al., 2005, 2008) using an analysis of variance (ANOVA) with two main effects (discrimination problem, subchronic treatment) and a problem × treatment interaction. The use of multiple discrimination problems in ED/ID test sessions permits non-EDS problems to serve as negative controls. Examination of enrichment effects on cognition was accomplished by using ANOVAs with two main effects (discrimination problem, enrichment treatment) and the problem × treatment interaction. Bonferroni *post hoc* analyses were used following a significant F test to assess significant group differences. Finally, we compared saline + EE and PCP + EE group differences in wheel running with an unpaired, 2-tailed *t* test. Then in order to explore the possible association between wheel running and cognitive performance, Pearson correlations between mean wheel running and performance on discrimination problems were calculated for both saline + EE and PCP + EE treatment groups. All alpha levels were set a priori at $p = 0.05$. All statistical analyses were performed using Prism 5.0c software (GraphPad, San Diego).

3. Results

3.1. Validity check on the formation of attentional set

One factor used in the interpretation of the EDS as a shift of attention is that the EDS problem is typically more difficult than the IDS problem in control subjects. In this experiment, control rats that received subchronic saline administration required a significantly greater number of trials (paired $t(7) = 2.41$, $p < 0.05$) to complete the EDS compared to the IDS (see Fig. 1).

3.2. Validity check of a selective PCP-induced cognitive deficits

We then examined effects of treatment group (SAL; PCP) across discrimination problems. There was a significant main effect of discrimination problem ($F(6, 84) = 11.10$, $p = 0.001$), a main effect of PCP treatment ($F(1, 14) = 6.69$, $p = 0.02$), but no significant discrimination problem by PCP treatment interaction ($F(6, 84) = 2.17$, $p > 0.05$). Post hoc analyses revealed that subchronic PCP-treated animals differed significantly from subchronic SAL-treated animals only on the trials to criterion for the EDS task ($t = 3.57$, $p < 0.01$). No significant PCP-induced impairment on trials to criterion was observed on any other discrimination problem (all $ts < 1.70$, $ps > 0.05$) (see Fig. 1).

3.3. Effects of environmental enrichment

We examined the ability of EE to attenuate the cognitive deficit in EDS function observed in animals treated with subchronic PCP administration. When PCP + EE animals were compared to PCP animals, analyses revealed a significant main effect of discrimination problem ($F(6, 84) = 20.51$, $p = 0.001$), a main effect of EE treatment ($F(1, 14) = 78.02$, $p < 0.001$), and a significant discrimination problem by EE treatment interaction ($F(6, 84) = 4.47$, $p < 0.001$). Post hoc analyses revealed PCP animals that received EE were significantly improved on EDS performance ($t = 7.22$, $p < 0.001$) and all three reversal problems (Rev1: $t = 3.25$, $p < 0.05$; Rev2: $t = 4.10$, $p < 0.01$; Rev3: $t = 4.58$, $p < 0.01$). In comparison, when the effects of EE were examined in animals treated with subchronic saline, analyses revealed a significant main effect of discrimination problem ($F(6, 84) = 2.58$, $p = 0.02$), a main effect of EE treatment ($F(1, 14) = 6.47$, $p = 0.02$), but no significant discrimination problem by EE treatment interaction ($F(6, 84) = 1.10$, $p > 0.05$). *Post hoc* analyses revealed EE treated saline animals did not differ significantly from saline control animals on any specific discrimination problem (SD $t = 1.05$; CD $t = 1.13$; Rev1 $t = 0.15$; IDS $t = 0.45$; Rev2 $t = 2.63$; EDS $t = 2.11$; Rev3 $t = 1.88$; all $p > 0.05$). Thus, EE significantly improved performance in PCP + EE animals and attenuated the PCP-induced deficit on EDS learning and also improved performance on reversal problems, whereas EE significantly improved overall performance in saline treated animals, but did not significantly improve performance on any specific discrimination problem (see Fig. 2).

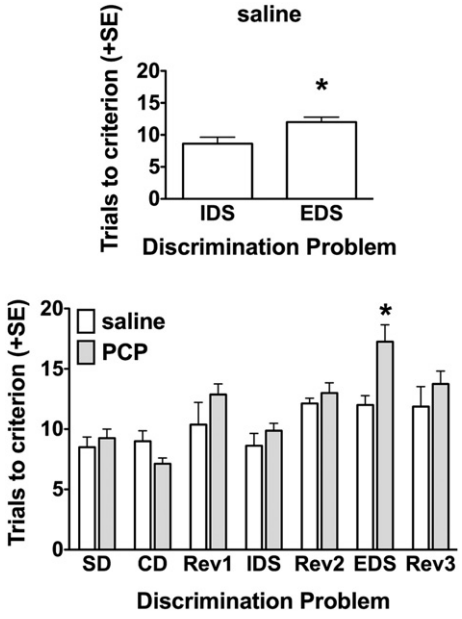

Fig. 1. Validity checks on formation of cognitive set and of selective EDS deficit. Top frame: trials to criterion are plotted as function of IDS and EDS discrimination problems for the subchronic saline treated group (SAL; white bars). Analyses revealed the EDS problem required significantly greater number of trials to reach criterion compared to the IDS problem (*$p < 0.05$), suggesting a cognitive set had been formed. Bottom frame: trials to criterion are plotted as a function of discrimination problem for both subchronic saline treatment (SAL; white bars) and subchronic PCP treatment (PCP; gray bars). Analyses revealed that PCP treatment produced a selective impairment in EDS performance only (*$p < 0.05$) compared to SAL treated control animals. PCP treatment did not impair performance on any other discrimination problem. Please consult Section 3 for details on statistical comparisons.

3.4. Wheel running

Animals in the saline + EE group had numerically greater levels of wheel running activity (mean = 2160 revolutions/session; SEM = 378.1) compared to the PCP + EE group (mean = 1354 revolutions/session; SEM = 100.2) (data not plotted), but this difference was trend level and did not reach statistical significance ($t(7) = 2.06$, $p = 0.08$). When associations between mean individual wheel running and performance on discrimination problems were calculated, no statistically significant relationships were observed (see Table 2). For PCP + EE animals, correlations ranged from $r = -0.54$ to 0.30, with all $ps > 0.17$, whereas for saline + EE animals the obtained r values range from $r = -0.68$ to 0.27, with all $ps > 0.06$. The strongest relationship was observed in saline + EE animals for Rev3 performance ($r = -0.68$, $p = 0.06$), suggesting a trend-level association such that increased wheel running resulted in improved performance (fewer trials to reach criterion) on the reversal problem that followed the EDS.

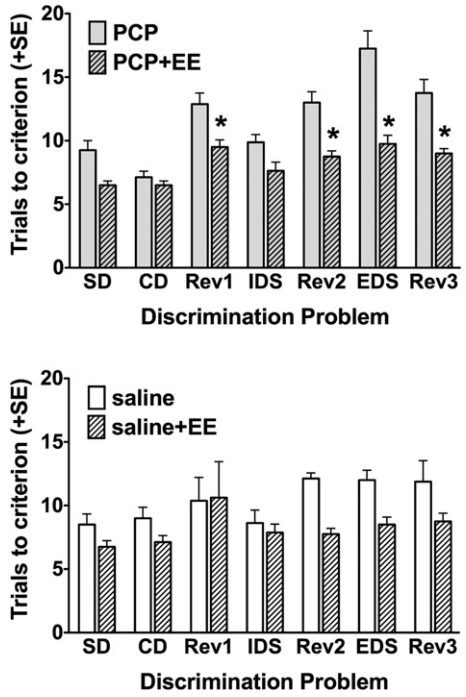

Fig. 2. Effect of environmental enrichment on cognitive performance. Top frame: trials to criterion are plotted as a function of discrimination problem for both subchronic PCP treatment (PCP; gray bars) and subchronic PCP treatment that also received environmental enrichment (PCP + EE; gray hatched bars). Analyses revealed a significant main effect of EE, with a significant improvement observed on selective discrimination problems (EDS, Rev1, Rev2, Rev3) (*$p<0.05$). Bottom frame: trials to criterion are plotted as a function of discrimination problem for both subchronic saline treatment (SAL; white bars) and subchronic saline treatment that also received environmental enrichment (SAL + EE; white hatched bars). Analyses revealed a significant main effect of EE, but no significant improvement observed on any selective discrimination problems. Please consult Section 3 for details on statistical comparisons.

4. Discussion

In the current study, we examined the cognitive effects of environmental enrichment on PCP-induced deficits in a rodent model of cognitive flexibility. We observed a number of novel findings. First, 30 days of limited (1 h/day) EE effectively ameliorated the PCP-induced impairment on EDS performance compared to saline treated control animals supporting our hypothesis that EE exposure would ameliorate the selective PCP-induced impairments in this rodent model of executive function. Second, we observed enhanced performance on reversal learning problems that were not initially impaired by the subchronic PCP treatment. Third, consistent with a great deal of literature (cf. Petrosini et al., 2009), we observed a significant main effect of EE-induced improvement in cognitive performance in both saline + EE and PCP + EE animals. These data may suggest that the simple behavioral mechanism of voluntary activity (Lautenschlager et al., 2008; Falls et al., 2010) in our EE procedure may be responsible for the general pro-cognitive effect, in addition to selective effects observed the PCP-treated animals. Our results are consistent with others (Karl et al., 2007) and suggest that even limited EE exposure can produce robust changes in cognitive performance. However we were not able to demonstrate a significant relationship between physical activity and cognitive performance on any specific discrimination problem examined in our study.

Table 2
Correlation *(p-level)* values between mean wheel running activity and performance on ED/ID discrimination problems.

	SD	CD	Rev1	IDS	Rev2	EDS	Rev3
Saline + EE	−0.19	0.26	−0.29	−0.22	0.19	−0.05	−0.68
	(0.66)	*(0.52)*	*(0.49)*	*(0.60)*	*(0.66)*	*(0.90)*	*(0.06)*
PCP + EE	0.30	−0.12	0.12	−0.54	0.25	0.29	0.26
	(0.47)	*(0.77)*	*(0.77)*	*(0.17)*	*(0.56)*	*(0.49)*	*(0.54)*

The impact of EE on reversal learning in PCP-treated animals was an interesting finding because PCP did not produce any impairment in reversal learning in the non-EE PCP group. Some improvement in performance was also observed in saline + EE animals on Rev2 and Rev3 problems, but these did not reach statistical significance. One possible explanation for these data is that significant decreases were observed in PCP + EE animals because PCP treated animals (non-EE) required more trials to reach criterion compared to saline-treated controls, and thus had a slightly poorer performance that was more easily improved by EE. Another complementary explanation is that the lack of significant improvement on reversal problems observed in the saline + EE animals might be due to a statistical floor effect such that saline animals were already near peak performance levels (a minimum of 6 trials required to reach criterion), and thus there was little opportunity to improve performance. Still a third possible explanation is that reversal deficits are most evident the first time the problem is encountered (Baxter, 2001). This might explain the comparable performance of saline and saline-EE animals on the Rev1 problem. Furthermore, previous research has demonstrated orbitofrontal cortex involvement in reversal learning (McAlonan and Brown, 2003), and thus may be differentially affected with regards to manipulations that improve (or impair) cognitive performance compared to medial frontal cortex (Barense et al., 2002; Rodefer and Nguyen, 2008).

One important aspect in understanding of the positive effects of enrichment on cognitive flexibility is the developmental time point when enrichment was administered. Animals in the present study received daily enrichment prior to, throughout, and following the pharmacological insult and as such, it is possible that enrichment may have played a role at any of these three different time points. First, enrichment may have enhanced cognition before the pharmacological insult occurred and made animals more resistant to insult. Secondly, enrichment may have blunted the impact of the insult itself, serving a protective role against cognitive impairment. Lastly, the continued enrichment exposure after PCP administration completion may have minimized any lasting effects of PCP during metabolism and washout. Any one or a combination of these mechanisms might be responsible for the cognitive enhancing effects observed following enrichment.

Results from the present experiment add to the growing literature regarding factors contributing to cognitive impairments associated with schizophrenia. A variety of manipulations in animal models of cognitive dysfunction have reliably produced impairment in the EDS portion of the attentional set-shifting task. These include surgical lesions of the medial prefrontal cortex and posterior parietal cortex (Birrell and Brown, 2000; Fox et al., 2003), drug manipulation via NMDAR blockade (Jentsch and Roth, 1999; Egerton et al., 2005) and cholinergic compounds (Chen et al., 2004), and neonatal administration of the neurotoxin methylazoxymethanol (Featherstone et al., 2007). One recent report has also suggested that social isolation in rats produced learning deficits (McLean et al., 2010). As only pharmacological mechanisms have previously demonstrated effectiveness in improving impairments in EDS performance, the utilization of behavioral manipulations like environmental enrichment as a means of cognitive enhancement adds to the current literature.

Identification of the mechanisms underlying the behavioral improvement was beyond the scope of these experiments. However, possible interpretations can be made from studies that have shown enrichment-induced modifications in monoaminergic systems implicated in cognitive flexibility. Changes in activation of monoamines influencing plasticity and learning, including dopamine, serotonin, and norepinephrine, have been observed in response to physical exercise and environmental stimulation (cf. van Praag et al., 2000). However, specific contributions of receptor subtypes within these

neurotransmitter systems have been more difficult to discern. Although haloperidol and most other atypical antipsychotics lack efficacy in reversing PCP-induced cognitive deficits in the ED/ID task (Rodefer et al., 2008), compounds with 5-HT6 receptor mediated activity have been demonstrated to improve cognition in the ED/ID task (Rodefer et al., 2008; Goetghebeur and Dias, 2009; Burnham et al., 2010; Nikiforuk et al., 2010).

Preclinical models have revealed effects of EE on several of the neurotransmitter systems proposed to be dysregulated in schizophrenia and cognitive impairment, including increased monoaminergic and NMDAR expression (Andin et al., 2007; Farmer et al., 2004; Naka et al., 2005). Mice with an ablated hippocampal CA1 NMDAR-1 subunit exhibit non-spatial memory deficits in the object recognition task, which was subsequently ameliorated by 2 months of daily EE sessions (Rampon et al., 2000). Yet some have reported lack of effects such that EE did not change NMDAR binding in the hippocampus or cortex (Gray et al., 2009) or alter NMDAR function in the rat barrel cortex (Lehohla et al., 2004). An alternative possible mechanism for the effects of EE has implicated the phospholipase C-beta 1 (PLC-beta1) signaling pathway as being both disrupted in schizophrenia and important for normal cognitive development (Hannan et al. 2001; Spires et al., 2005; McOmish et al., 2007). The use of EE treatment has been reported to attenuate behavioral impairments that were associated with schizophrenia (McOmish et al., 2007). Moreover, sensory enrichment has been demonstrated to increase physiological plasticity, even following selective cholinergic deficits (Percaccio et al., 2007).

In considering these experiments in sum, there were several strengths. First, we utilized a behavioral task that is sensitive for the evaluation of executive function in rodents (Birrell and Brown, 2000; Fox et al., 2003). Second, we produced a selective PCP-induced deficit in executive function that mirrors neuropsychological impairments observed clinically in schizophrenia patients. Third, our validation check on the differential acquisition of EDS vs. IDS in control subjects was consistent with previous experiments both from our lab (Rodefer et al., 2005, 2008) and other labs using the same (Barense et al., 2002; Fox et al., 2003) and different stimuli (Dias et al., 1996, 1997, McLean et al., 2010). Fourth, the validation check on deficit production revealed a robust and selective EDS deficit observed in PCP-treated animals, mirroring previous results from both our laboratory (Rodefer et al., 2005, 2008) and others (Egerton et al., 2005). Thus, these data clearly supported the utility and validity of our model and support the continued reliability of this pharmacological regimen in detecting clinically relevant treatments for cognitive enhancement.

Methodological consistency appears to be one likely future target for improvement and standardization of environmental enrichment studies. There are no currently accepted standards for environmental enrichment designs, and researchers often include various factors (e.g., dietary changes, toys, social interaction) in a non-standardized manner that do not permit clear determination of significant contributions for individual components (Nithianantharajah and Hannan, 2006). We used standardized chambers and running wheels, and a consistent pattern of diet supplementation and enriching object availability. Standardization of enrichment paradigms or parametric evaluations may improve reproducibility of results. However, given the lack of understanding on the role of each component we used in our enrichment protocol, it is possible that pro-cognitive effects could be due solely to aerobic exercise, or any other individual component common in most enrichment paradigms. Thus, further investigation of this issue is warranted.

In conclusion, these data provide the first evidence of the attenuation of cognitive deficits induced by NMDA blockade by EE exposure. These results add to the growing literature investigating EE as an approach to dissecting the complex interactions involved in the development of cognitive dysfunction associated with neuropsychiatric disorders. Treatments aiming to mimic the ameliorative effects of enrichment on this model of cognitive impairment may be a useful tool in the development of new clinically therapeutic strategies.

Conflict of interest statement

JSR has received research materials or support from Bristol Myers Squibb, H. Lundbeck A/S, Janssen Pharmaceutical, and Roche during the past 3 years. The authors declare no other conflicts of interest (actual or potential).

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