Life Sciences 218 (2019) 139–146

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Repeated administration of methylphenidate produces reinforcement and downregulates 5-HT-1A receptor expression in the nucleus accumbens

Tabinda Salman[a,*], Shazia Nawaz[a], Rizwana S. Waraich[b], Darakhshan J. Haleem[a]

[a] Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

[b] Pathobiochemistry and Molecular Physiology Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

ARTICLE INFO

Keywords:
Methylphenidate
Conditioned place-preference
Addiction
Cognition
qRT-PCR

ABSTRACT

Aims: Methylphenidate (MPD) widely prescribed for the treatment of attention deficit hyperactivity disorder (ADHD), is a psychostimulant and can produce addiction in patients treated with it. In view of growing increase in the use of drug by general population as a cognitive enhancer, the present study is designed to investigate reinforcing and cognition enhancing effects of MPD in rats. Associated changes in serotonin-1A receptor expression are investigated as a potential molecular mechanism involved.

Methods: Learning acquisition and memory retention in Morris water-maze test were used to assess cognitive effects of MPD. Reinforcing effects were evaluated in conditioned place-preference (CPP) paradigm. The expression of 5-hydroxytryptamine (5-HT; serotonin)-1A receptor in the nucleus accumbens and prefrontal cortex of repeated MPD treated animals was determined by qRT-PCR.

Findings: Lower doses (0.5 and 2.5 mg/kg) of MPD enhanced learning acquisition and memory retention but higher doses (5 mg/kg) impaired these. The drug administered repeatedly at a dose of 2.5 mg/kg was reinforcing in CPP test, but sensitization like effects of this dose were only transient. These animals tested in water-maze test exhibited improved memory retention but no effect occurred on learning acquisition. The expression of 5-HT-1A receptor was markedly attenuated in the nucleus accumbens; attenuation in the prefrontal cortex was not significant.

Significance: The findings suggest that clinically relevant doses of MPD can produce drug addiction, but effects of the drug on memory retention are retained. A downregulation of 5-HT-1A receptor in the nucleus accumbens seems important in the reinforcing effects of MPD.

1. Introduction

Methylphenidate (MPD) is widely prescribed for the treatment of Attention-deficit hyperactivity disorder (ADHD) since 1957 [1]. ADHD is a disorder characterized by inattentiveness, hyperactivity and impulsivity [2]. Children are more affected by this disease and the worldwide prevalence is about 5.3% [3–5]. The drug is also used by general population, especially college students to improve academic and work related performances [6,7]. Evidence suggests that therapeutic or non therapeutic use of MPD results in drug overuse and addiction [8].

ADHD is a multi-factorial highly heritable disorder and several animal models of ADHD currently exist [9,10]. These models define the fundamental aspects of ADHD behavior such as impulsivity, hyperactivity and inattention [11,12]. Although the drug is not necessarily used in clinically relevant doses by general population, a growing increase in MPD use and over-use has been seen in general population where the drug is potentially used for its nootropic effects [6]. Over-use of the drug has also been seen in ADHD patients using therapeutic doses of MPD [7]. The present study is designed to assess addictive effects of clinically relevant doses of MPD in normal rats. Whether these doses can improve learning and memory in normal rats is also tested.

Dose related effect of MPD on learning and memory are determined in Morris water-maze test. Drugs which produce addiction are rewarding, reinforcing and elicit behavioral sensitization [13]. A dose (2.5 mg/kg), which is a memory enhancing and clinically relevant too [14], is administered repeatedly in conditioned place-preference paradigm (CPP) to determine its potential reinforcing and sensitization like effects.

MPD is thought to produce its pharmacological effects by inhibiting

* Corresponding author.
E-mail addresses: tabinda.salman89@gmail.com (T. Salman), djhaleem@uok.edu.pk (D.J. Haleem).

https://doi.org/10.1016/j.lfs.2018.12.046
Received 24 August 2018; Received in revised form 18 December 2018; Accepted 27 December 2018
Available online 27 December 2018

dopamine and noradrenaline reuptake [15]. The resulting increase in the synaptic availability of dopamine and noradrenaline can reduce symptoms of ADHD [15,16]. The drug induced increase in catecholamine neurotransmission within the prefrontal cortex is thought to improve cognition [17] while an increase in extracellular dopamine in the nucleus accumbens produces reinforcement to lead to drug addiction [18].

Evidence suggests that serotonin (5-hydroxytryptamine; 5-HT) plays an important role in cognition as well as addiction. These effects of serotonin are mediated via the activation of 5-HT-1A receptors, which modulate dopamine neurotransmission in the nucleus accumbens as well as the prefrontal cortex [19]. Thus co-administration of buspirone, an agonist at 5-HT-1A receptor, inhibited apomorphine as well as morphine-induced addiction [20–22]. We hypothesized that due to agonist activity for 5-HT-1A receptors [23,24], MPD may have little or no reinforcing effects. Therefore, in the present study, reinforcing effects of MPD and 5-HT-1A receptor expression in the nucleus accumbens are determined.

Prefrontal cortex is known to have an important role in learning and memory [25]. It is also involved in the formation of addiction related memories [26]. 5-HT-1A receptors are highly expressed in the prefrontal cortex [27] and activation of these postsynaptic receptors can interfere with memory retrieval [28]. Memory retention in animals treated repeatedly with MPD and its association with 5-HT-1A expression in the prefrontal cortex is also determined.

2. Methodology

2.1. Animals

Animals were housed and handled according to the strict guidelines of 'Guide for the care and use of laboratory animals', The National Academies Press, Washington D.C, USA and the Institutional Animal Ethics Committee (IAEC; Animal study protocol no. 2015-0014). Although, a precise relationship between the age of laboratory rats and human is still a matter of debate, male albino rats, which were not sexually mature (< 6 weeks of age; 4–5 weeks old), weighing 180–220 g were used in the present study. The animals provided by the animal house, ICCBS, University of Karachi were housed individually and kept under 12 h light dark cycle (a light turned on at 8:00) and controlled room temperature (24 ± 2 °C) with access to tap water and cubes of standard rodent diet 5 days before the start of the experiment. Rats were acclimatized to various handling procedures so as to reduce the psychological stress of the environment.

2.2. Drugs

Methylphenidate (Ritalin, Novartis) was purchased locally and administered orally. The tablets were pulverized and dissolved in distilled water. Before each experiment, drug solution was freshly prepared. A measured volume of it (according to dose) was administered orally via syringe connected gavages as described before [29]. The animals were handled carefully to avoid any distress or gavages related injury till the drug was totally taken and not expelled out.

It has been shown that oral doses of MPD ≤ 3 mg/kg produce plasma drug level in rodents which is equivalent to the clinically effective doses in humans [30–32]. The drug was administered at a dose of 0.5, 2.5 and 5 mg/ml/kg in the first experiment (dose related study). It was administered at a dose of 2.5 mg/ml/kg repeatedly in the second experiment. MPD is rapidly absorbed after oral administration with the peak plasma concentration achieved in an average of 1 h [33–35]. The experiments on cognition and reinforcing effects of MPD were designed accordingly.

2.3. Experiment 1 - dose-related effects of MPD on water-maze performance

Twenty four animals were used to determine dose related effects of MPD on learning acquisition and memory retention in water-maze test. The rats were divided randomly into four groups, each containing six animals. MPD was administered, orally, in doses of 0.0, 0.5, 2.5 and 5 mg/kg, at 09:00 h–11:00 h, immediately before training in Morris water-maze.

2.3.1. Water-maze test

The apparatus and procedure was essentially same as described before [29]. The maze, a white circular pool, 90 cm in diameter and 60 cm high, was used. It was filled with water, made opaque with milk, to a depth of 30 cm. The apparatus placed in an experimental room was surrounded with constant visual cues (window, cabinets, equipment etc.). The maze was divided virtually into four equal quadrants (north, south, east and west). In the center of the north quadrant a square platform (10 × 10 cm) was placed at a height 2 cm below the surface of water.

Acquisition and retention of memory were assessed as the latency time to locate the hidden platform. The procedure consisted of two phases: the training phase and the test phase. In the training phase, all animals had successive three training trials, one from each quadrant (other than the quadrant with hidden platform), to locate the platform. Cut-off time was 2 min for each session. If the rat succeeded, it was allowed to stay on it for 10 s and if failed, then guided towards the platform. In the test phase, acquisition and retention of memory were tested. The platform was placed in the north quadrant and animals were entered from a single position (opposite quadrant to the north) to locate the hidden platform (cut-off time = 2 min). The acquisition and retention of memory were assessed respectively after 2 and 24 h of the training.

2.4. Experiment 2- effects of repeated administration of MPD

Sixteen rats were subjected to a 13-day procedure of place conditioning and preference test as shown in Table 1. The rats were randomly divided into two groups each containing eight animals. MPD was orally administered at a dose of 2.5 mg/kg, while the control group was treated with water. This dose was selected from the results of previous experiment of dose response curve and administered in CPP paradigm as shown in Table 1 and described below in the Section 2.4.1.2, on drug conditioning.

Table 1
Scheme for repeated administration of drug and water thorough out the experiment.

Treatment days	Pre-conditioning	Conditioning phase												Post-conditioning	Training & learning acquisition	Memory retention	Decapitation
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control (water)	N	W	W	W	W	W	W	W	W	W	W	W	W	N	W	W	W
Treatment (methylphenidate)			D		D		D		D		D		D		D		D

{*W = water, *D = drug (2.5 mg/kg methylphenidate), N* = No drug/water}.

2.4.1. Reinforcing effects in CPP-paradigm

The CPP test was conducted to monitor reinforcing effects of repeated administration of MPD. The test was carried out in a three compartment place-preference apparatus of unbiased design as described before [21]. The two end-compartments (26 × 26 × 26 cm) were separated by guillotine sliding doors and a shuttle compartment in the middle (10 × 26 × 26 cm). The end-compartments provided different environment perception with one compartment having black horizontals strips (side walls) and grid rod floor while the other compartment having vertical strips of black color and meshed floor made up of stainless steel. The middle shuttle compartment had the smooth floor and transparent guillotine sliding doors. A video recording system was placed appropriately to monitor all the sessions of place conditioning. The test was conducted to determine preconditioning place preference, followed by drug conditioning after which post conditioning place preference was determined. Motor activity was monitored during the conditioning phase to assess behavioral sensitization.

2.4.1.1. Pre-conditioning place preference. As shown in Table 1, on the pre-treatment day (day 0), animals exposed individually to the CPP apparatus were allowed to explore the apparatus and establish preconditioning responses and monitor any probable bias for either compartment. Animals were entered through the central shuttle compartment and after 10 s, sliding doors of both end compartments were raised and animals allowed to move freely and explore all three compartments for 10 min (at 9:00 h–10:00 h). The time spent in both end compartments (preference) was recorded. Animals showing no preference for either compartment were included in the study.

2.4.1.2. Drug conditioning. Animals went through drug conditioning (one session per day) for 12 days (Table 1). During drug conditioning, an animal was confined with either the horizontal or vertical strip compartment after entering from the shuttle compartment. On day 1, 3, 5, 7, 9 and 11 animals were treated with water orally (at 9:00–12:00 h) and placed in the 'non-drug' associated compartment (horizontal strips) for 30 min. On the other days i.e. 2, 4, 6, 8, 10 and 12, control animals were treated with water while test animals were treated with MPD (at 9:00–12:00 h) and placed immediately in the 'drug' associated compartment (vertical strips) for 30 min.

2.4.1.3. Post-conditioning test. The post-conditioning test was conducted on day 13 at 09:00 h–10:00 h. The test was conducted essentially in the same way as preconditioning test. An animal was placed in the shuttle compartment and guillotine sliding doors of both the end-compartments were raised. The animal was allowed to move freely in all compartments for 10 min. Time spent in 'drug' and 'non-drug' associated compartment was monitored to determine place preference as described before [21].

2.4.2. Effect of repeated administration of MPD on motor activity

The effects on motor activity were scored during conditioning phase (at 09:00 h–12:00 h) via video recording device. The activity was scored on drug treatment day as well as on water treatment day. As described above control animals were treated with water on drug treatment day as well as on water treatment day. Motor activity in control animals was also monitored every day. The activity was scored as the numbers of compartment crossings (both horizontals as well as vertical) starting 5 min post-injection for a period of 10 min.

2.4.3. Effect of repeated administration of MPD on learning acquisition and memory retention

The effects on learning and memory in rats treated repeatedly with MPD were determined on day 14 and day 15. Morris water-maze and the procedure described in experiment 1 were used to determine performance. On day 14 (Table 1), MPD or water was administered to, respectively, test and control group. Immediately after the administration animals were trained to locate the submerged escape platform in the water-maze. The training session continued from 9:00 h to 11:00 h. Following the training session, learning acquisition and memory retention were monitored as the latency to mount the escape platform. Test for learning acquisition was conducted at 13:00 h to 14:00 h and that for memory retention at next day (day 15) at 9:00 h to 11:00 h.

2.5. Collection of brain samples and gene expression analysis of serotonin-1A receptor

The experiment for collecting samples for 5-HT-1A receptor expression was performed on day 16 (Table 1). The animals treated with MPD or water at 09:00 h–11:00 h were decapitated 1 h post treatment. Brains taken out immediately were micro-dissected to collect the nucleus accumbens and prefrontal cortex as described earlier [36]. The samples were stored at −80 °C for determining the expression of serotonin-1A receptor.

2.5.1. RNA isolation and reverse transcription

The samples were homogenized in TRIzol® reagent (Ambion, Life technologies) and total RNA were isolated according to the protocol described by the manufacturer. Photometric measurements of total RNA concentrations were carried out at 260 nm using a NanoDrop (ThermoScientific Multiskan GO). Reverse transcription was performed with 1 μg of total RNA from each sample via RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) with random primers in Master cycler proS (Eppendorf). The guidelines provided by the manufacturer were followed during the procedure.

2.5.2. Quantitative Real-time polymerase chain reaction (qRT-PCR)

Specific primers of serotonin (5-HT)-1A and β-actin designed and synthesized by Penicon Pharmaceuticals were used (Table 2). PCR products were quantified fluorimetrically using SYBR green (SG) dye. This cyanine dye was used to bind double stranded DNA. The resulting DNA-dye complex, which absorbs blue light (λmax = 497 nm) and emits green light (λmax = 520 nm), helped quantify DNA [37].

Real-time PCR was done in Stratagene Mx3000P (Agilent technologies, USA) with Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix. Thermal amplification cycling conditions included an initial de-naturation step at 95 °C for 10 min and the run for 40 cycles for 15 s at 95 °C, annealing at 60 °C for 30 s and final extension at 72 °C for 30 s. Data were acquired and analyzed using comparative CT method (MxPro Software). β-Actin mRNA was used as an internal loading control, co-amplified with 5-HT-1AR mRNA. The levels of the 5-HT-1AR mRNA were normalized by β-actin.

Table 2
Primer sequences with annealing temperatures and product size.

PCR Primers	Sequences	Annealing temperature (°C)	Product size (bp)
Beta actin (F)	5′-ACCCACACTGTGCCCATCTA	58.5	285
Beta actin (R)	5′-CGGAACCGCTCATTGCC	57.1	
5-HT-1A receptor (F)	5′-CCCCCCAAGAAGAGCCTGAA	59.4	335
5-HT-1A receptor (R)	5′-GGCAGCCAGCAGAGGATGAA	60.1	

2.6. Determining food intake and body weight changes

Food intake (g) was monitored by taking the difference of food given on day 1, and food left at the end of experiment i.e. on decapitation day. Pre-weighed food was provided in the built-in stainless steel hopper of the cage. Body weights were also monitored on starting day and on decapitation day. Percentage change in the body weight was calculated as (Body weight on decapitation day / Body weight on start day) × 100.

2.7. Statistical analyses

All results are presented as means ± S.D. Behavioral data were analyzed by two-ways ANOVA (factor 1, drug; factor 2, repeated measure) followed by Tukey's post-hoc test. Data on food intake, body weight change and expression studies were analyzed by Mann-Whitney *U* test. Results with p values < 0.05 were considered statistically significant.

3. Results

Fig. 1 shows dose related effects of MPD on learning acquisition and memory retention in water-maze test. Latency time to reach and mount the escape platform was taken as a measure of learning and memory. Because the same animals were used in learning acquisition and memory retention, two-way ANOVA (factor 1 = drug, factor 2 = repeated measure) with repeated measure design was used. The analysis showed significant effect of MPD ($F = 111.12$; $df = 3,20$; $p < 0.01$), repeated measure ($F = 255.697$; $df = 1,20$; $p < 0.01$) and significant ($F = 9.274$; $df = 3,20$; $p < 0.01$) interaction between MPD and repeated measure. Post-hoc test showed that latency time to reach the escape platform, in learning acquisition as well as in memory retention phase, was smaller in animals treated with low doses (0.5 mg/kg, 2.5 mg/kg) MPD treated than water treated animals. Conversely, animals treated with 5.0 mg/kg MPD exhibited an increase in the latency time compared to water treated animals or low doses (0.5 and 2.5 mg/kg) MPD treated animals. The results suggest that low doses of MPD improve learning acquisition as well as memory retention while higher doses impair it.

Fig. 2 shows reinforcing effects of 2.5 mg/kg MPD in CPP paradigm. Data on pre and post conditioning values of time passed in drug paired compartment, analyzed by two-way ANOVA (repeated measure design) showed significant effect of MPD ($F = 15.367$; $df = 1,14$; $p < 0.01$), repeated measure ($F = 11.430$; $df = 1,14$; $p < 0.01$) and significant ($F = 7.274$; $df = 1,14$; $p < 0.05$) interaction between MPD and repeated measure. Post hoc analysis showed that pre- and post-conditioning values of time spent in drug paired compartment were not different in water treated animals. MPD treated animals exhibited an increase in post- than pre-conditioning values of time spent in drug-paired compartment. Post-conditioning values of time spent in drug-coupled compartment was also greater in MPD than water treated animals. The results suggest that doses of MPD which improve cognition are reinforcing.

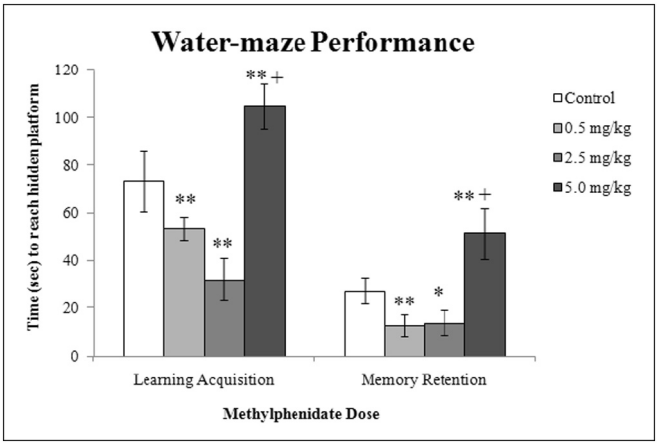

Fig. 1. Dose related effects of MPD on learning acquisition (2 h after training) and memory retention (20 h after learning acquisition) as monitored in Morris water-maze. Values are means ± SD (n = 6). Significant differences by Tukey's test: **p < 0.01, *p < 0.05 from water treated animals; +p < 0.01 from lower doses of MPD treated animals following two-way ANOVA repeated measure design.

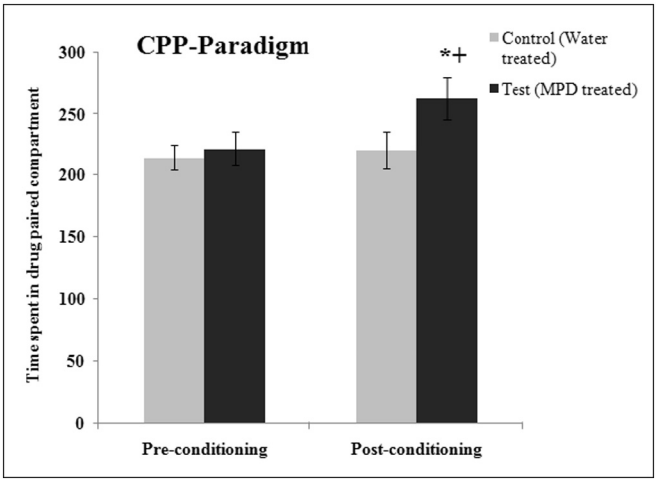

Fig. 2. Effect of MPD (2.5 mg/kg) on pre- and post-conditioning values of time passed in the drug compartment. Values are means ± SD (n = 8). Significant differences by Tukey's test: *p < 0.01 from respective water treated animals; +p < 0.01 from respective preconditioning values following two-way ANOVA repeated measure design.

Fig. 3 shows motor activity in controls and test animals during conditioning phase. The activity on non drug treatment day is shown in Fig. 3A, while the activity on drug treatment day is shown in Fig. 3B. Data of water treatment days (day 1, 3, 5, 7, 9, and 11) analyzed by two-way ANOVA repeated measure design showed significant effect of MPD ($F = 74.671$; $df = 1,10$, $p < 0.01$), repeated measure ($F = 5.205$; $df = 5,70$; $p < 0.01$) and significant ($F = 6.107$; $df = 5,70$; $p < 0.01$) interaction between MPD and repeated measure. Post-hoc analysis showed significant increase in motor activity in MPD treated than water treated animals on day 3, 5 and 7. The MPD treated animals exhibited greater motor activity on day 5 than on day 1. Differences on other treatment days were not significant. Results suggest that motor activity enhancing effects of MPD also occur 24 h after the drug administration.

Data on drug treatment days (day 2, 4, 6, 8, 10 and 12) analyzed by two-way ANOVA repeated measure design showed significant effects of MPD ($F = 178.779$; $df = 1,10$, $p < 0.01$) and repeated measure ($F = 6.108$; $df = 5,70$; $p < 0.01$). Interaction between MPD and repeated measure was not significant ($F = 1.255$; $df = 5,70$; $p > 0.05$). Post-hoc analysis showed significant increase ($p < 0.05$) in motor activity in MPD than water treated animals after every administration. The activity in MPD treated animals on day 6 i.e. following 3rd MPD administration was greater than the values observed following 1st MPD administration suggesting sensitization like effects. However, these effects did not persist following subsequent administration. Results suggest that sensitization like effects following repeated MPD administration are only transient and do not persist on further administration.

Fig. 4 shows learning acquisition and memory retention in repeated water (control) and MPD treated (test) animals. Data analyzed by two-way ANOVA (repeated measure design) showed significant effect of MPD ($F = 24.598$, $df = 1,14$, $p < 0.01$) and significant ($F = 5.098$, $df = 1,14$, $p < 0.05$) interaction between MPD and repeated measure.

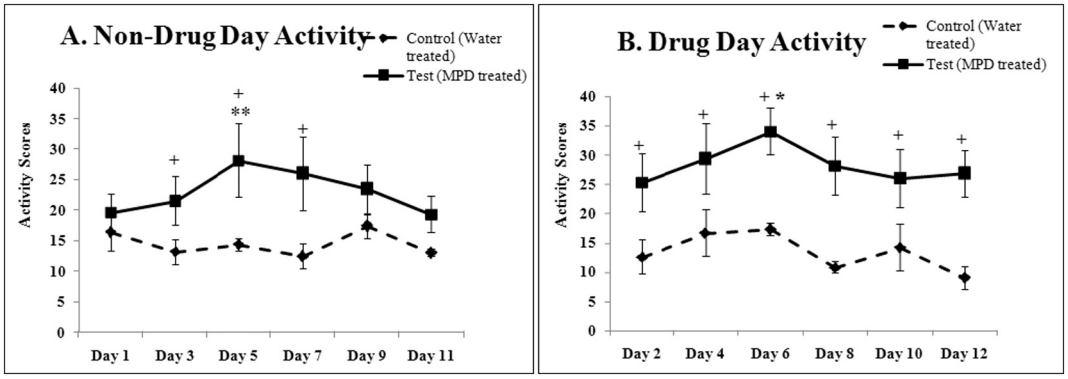

Fig. 3. Effects of MPD (2.5 mg/kg) on motor behavior monitored on water treatment days (Day 1, 3, 5, 7, 9, 11) and drug treatment days (Day 2, 4, 6, 8, 10, 12). Values are means ± SD (n = 8). Significant differences by Tukey's post hoc test: *p < 0.05 from respective water treated animals; +p < 0.01 from respective first administration (i.e. from day 1 in nondrug day activity and day 2 in drug day activity), following two-way ANOVA repeated measure design.

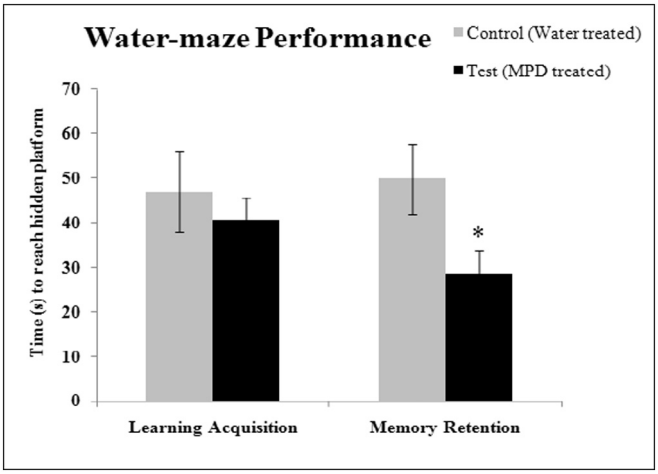

Fig. 4. Effect of repeated administration of MPD (2.5 mg/kg) on learning acquisition and memory retention in water-maze test. Values are means ± SD (n = 8); Significant differences by Tukey's post hoc test: *p < 0.01 from respective water treated animals (memory retention) following two-way ANOVA repeated measure design.

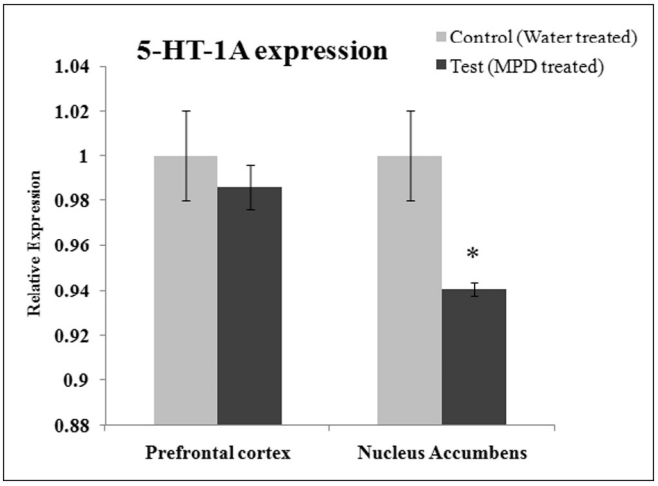

Fig. 5. Effect of MPD (2.5 mg/kg) on 5-HT-1A receptor expression in the prefrontal cortex and nucleus accumbens by qRT-PCR. Values are means ± S.D. (n = 8). Significant differences by Mann-Whitney *U* test: *p < 0.01 from water treated animals.

While the effects of repeated measure (day) were not significant (F = 1.947, df = 1,14). Post-hoc analysis showed that latency time to reach the escape platform was not different in MPD than water treated animals during learning acquisition phase. The latency time during memory retention phase was significantly (p < 0.01) smaller in MPD than water treated animals. Results suggest that repeated administration of MPD continues to improve memory retention but tolerance occurs in the effects of MPD on learning acquisition.

Fig. 5 shows effect of repeated administration of MPD on 5-HT-1A receptor expression in the prefrontal cortex and nucleus accumbens. Mann-Whitney *U* test revealed significant decrease (p < 0.01) in 5-HT-1A receptor expression in the nucleus accumbens of MPD than water treated animals. Similar decreases of 5-HT-1A receptor expression in the prefrontal cortex were not significant (p > 0.05).

Fig. 6 shows effect of repeated administration of MPD on changes in body weight and food intake. Data analyzed by Mann-Whitney *U* test showed that the effects on body weight changes and food intake were not significant (p > 0.05).

4. Discussion

The aim of the present study was twofold. Firstly, we wanted to know whether doses of MPD equivalent or greater than clinically recommended doses improve memory in normal rats. Another aim was to monitor memory retention in animals exhibiting reinforcement to drug and determine its association with 5-HT-1A receptor expression. We found that higher doses of MPD (5 mg/kg) impaired memory, but lower doses (0.5 and 2.5 mg/kg), improved memory. Because memory enhancing effects were greater in rats treated with 2.5 mg/kg than 0.5 mg/kg MPD, the dose producing a greater effect on memory enhancement was selected for repeated administration and monitor tolerance, if any, in these effects on repeated administration.

Our finding that higher doses of MPD impair water-maze performance suggests that doses of MPD for the treatment of ADHD should be carefully evaluated. The finding that a lower doses of MPD which improve memory is also reinforcing and this effect of MPD is associated with a downregulation of 5-HT-1A receptors in the nucleus accumbens supports the notion that together with other psychostimulatnts [14] and morphine [19,20], 5-HT-1A receptor dependent mechanism is also important in MPD addiction. Moreover, we show that the downregulation of 5-HT-1A receptors selectively occurs in the nucleus accumbens and a similar downregulation does not occur in the prefrontal cortex, a region of the brain associated with learning and memory. Our results that the effects of MPD on improving memory retention persist in animals exhibiting reinforcement for drug suggest that MPD abuse is not related with tolerance in its effects on memory retention.

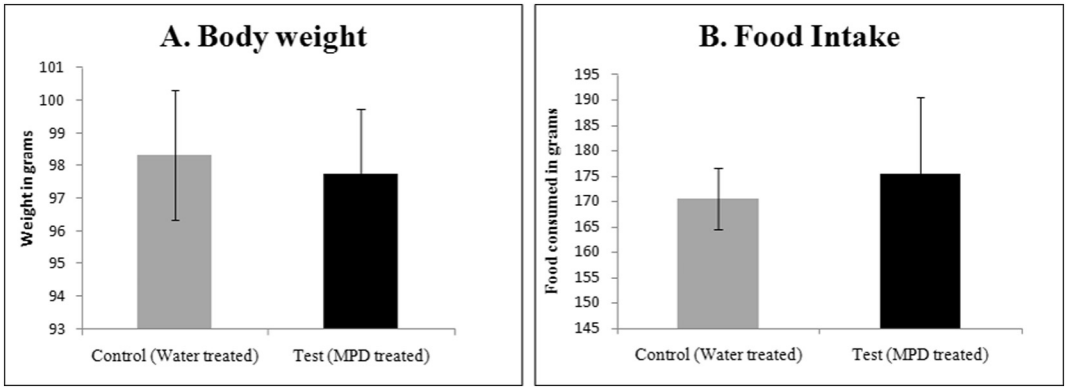

Fig. 6. Effect of MPD (2.5 mg/kg) on body weight and food intake. Values are means ± S.D. Differences by Mann-Whitney *U* test were not significant.

4.1. Dose related memory enhancing and impairing effects of MPD

Previously, it has been shown that oral administration of MPD in doses of 3.0 mg/kg improves radial maze learning and performance in preadolescent rats [38]. It has been also shown that low doses of MPD improve learning acquisition, memory retention and reconsolidation in Morris water-maze test [29]. Reports on the effects of higher doses of MPD on learning and memory are not very consistent as an enhancement as well as reported to occur depending upon the model used [39,40].

The memory enhancing effects of MPD are thought to occur because of its ability to inhibit high affinity reuptake of catecholamines and facilitate dopamine and nor-adrenaline neurotransmission [17,41]. On the other hand, memory impairment in high dose MPD treated animals is often linked with the excessive increase in the synaptic concentration of dopamine and noradrenaline [32,40,42,43]. In addition, MPD has agonist activity for 5-HT-1A receptors [23]. Activation of postsynaptic 5-HT-1A receptors also facilitates dopamine and noradrenaline neurotransmission and the release of these catecholamines in the nucleus accumbens and prefrontal cortex is enhanced [19,44]. Evidence suggests that optimal increases in synaptic dopamine and noradrenaline preferentially activate D1 and alpha-2 receptors, resulting in an enhancement of information flow and strengthening of memory [67]. On the other hand, excessive increase in synaptic dopamine and noradrenaline at higher doses of MPD activates dopamine D2 like receptors and noradrenergic alpha-1 and beta receptors resulting in the weakening of information flow and memory retention [45,67]. The present results on memory enhancing effects of low doses MPD are therefore explainable in terms of optimal increases in catecholamine neurotransmission due to inhibition of high affinity reuptake and activation of postsynaptic 5-HT-1A receptors. Excessive increase in catecholamine neurotransmission can, however, impair learning acquisition and memory retention observed in high dose MPD treated rats.

4.2. Reinforcing effects of MPD

4.2.1. Relationship with 5-HT-1A receptor expression in the nucleus accumbens

The CPP method is widely used to monitor the reinforcing properties of drugs of abuse [20,21,46,68]. Drugs of abuse are rewarding and reinforcing and elicit preference for drug associated compartment in CPP-test [14,20,47,48,69]. Repeated administration of these drugs also produces sensitization. In a study comparing the reinforcing effects of low and high dose of MPD, it has been reported that high (10 mg/kg) but not low (3 mg/kg) doses of MPD, paired 4 times with an end compartment in CPP paradigm are reinforcing [49]. We report that slightly smaller doses (2.5 mg/kg) of MPD, paired 6 times with an end compartment in CPP paradigm are also reinforcing, but produce only transient sensitization like effect. The results tend to suggest that doses of MPD which improve memory are reinforcing and strategies to alleviate the reinforcing effects are needed for improving therapeutic use of MPD.

Rewarding and reinforcing effects of drugs of abuse are processed through an increase in dopamine neurotransmission in the mesolimbic pathway [14,50]. The release of dopamine in the nucleus accumbens, a terminal region of mesolimbic pathway, elicits reward perception which predisposes to addiction [14,19,51]. Conversely, a decrease in dopamine neurotransmission via the nucleus accumbens, particularly during drug withdrawal, produces reinforcement for drug use. In view of role serotonin 1A receptors in inhibiting the reinforcing effects of drugs of abuse [20,52], it is important to note that activation of postsynaptic 5-HT-1A receptors in the nucleus accumbens is expected to increase neurotransmission via mesolimbic pathway [14,19]. Previously we have shown that a decrease in serotonin levels in the nucleus accumbens resulting in reduced dopamine neurotransmission via mesolimbic pathway is involved in the reinforcing effects of apomorphine [21]. A downregulation of 5-HT-1A receptors, as observed in the present study, in the nucleus accumbens is also expected to decrease dopamine neurotransmission via reward pathway. Together, the present and our previous studies [14,21] tend to suggest that activation of postsynaptic 5-HT-1A receptors in the nucleus accumbens produces an excitatory influence on dopamine neurotransmission via reward pathway. Long term exposure to drugs of abuse weakens this effect of 5-HT on dopamine neurotransmission to lead to reinforcement and drug abuse.

4.3. Memory enhancing effects of MPD following repeated drug exposure: relationship with 5-HT-1A receptor expression in the prefrontal cortex

Although repeated administration of MPD improves memory reconsolidation [29], tolerance in its cognitive effects [53,54] has been also reported. The present study shows that repeated exposure to nootropic doses of MPD produces tolerance in learning acquisition but not memory retention. The prefrontal cortex plays an important role in cognitive behaviors such as spatial memory, and decision making [55,56]. It is interesting to see that, unlike nucleus accumbens, a downregulation of 5-HT-1A receptors is not produced in the prefrontal cortex of repeated MPD treated rats. Because memory retention is also not impaired in these rats, the findings tend to support a role of 5-HT-1A receptor mediated control of information flow via the prefrontal cortex in memory retention [57].

The 5-HT-1A receptors are highly expressed in the prefrontal cortex [27]. Activation of 5-HT-1A receptors in this brain region facilitates dopamine neurotransmission via mesolimbic reward pathway [19] and interferes with memory retrieval [28]. Therefore, to explore the mechanism underlying cognitive effects of repeated MPD treatment in rats exhibiting reinforcement for drug, we focused on 5-HT-1A expression in the prefrontal cortex. In this context, an upregulation of these receptors

would be expected to enhance dopamine neurotransmission [19] and weaken memory circuit [28]. Our results that no effect occurs on 5-HT-1A expression in the prefrontal cortex are relevant that effects of MPD on memory retention continue even after the expression of drug reinforcement.

Together with the prefrontal cortex, 5-HT-1A receptors are also highly expressed in the hippocampus, and activation hippocampal 5-HT-1A receptors are also known to play important role in learning and memory [26,58]. Studies on the 5-HT-1A receptor expression in the hippocampus of repeated MPD treated rats could help to further expand our knowledge of the relationship of 5-HT-1A receptors with addiction and cognition.

4.4. Effects of MPD on food intake and body weight

Chronic use of particularly high doses MPD has been seen to be associated with appetite suppression [59–61]. Conversely, discontinuation of MPD after chronic use is reported to produce a significant increase in body weight as well as food consumption [62]. On the other hand, studies linking addiction with obesity provides evidence that low brain dopamine activity predisposes to addiction as well as obesity [63]. Moreover, the appetite suppressant effects of MPD are not produced in obese and overweight adults who met the diagnostic criteria of food addiction [64]. In general, low doses of MPD if used for a smaller period of time have been shown to produce no effect on appetite [65]. Only few preclinical studies have addressed the effects of MPD on food intake. These studies show a dose dependent reduction in body weight [66] or both an enhancement and suppression depending on the duration of drug administration [53]. We show that administration of low dose of MPD on alternate days for short period produces no effect on food intake. It is however possible that the same dose if administered daily for a longer time can alter food intake and body weight.

5. Conclusion

In conclusion, the present study shows that therapeutic doses of MPD should be carefully evaluated as higher doses can impair cognitive performance. We show that a dose of MPD which improves memory produces no effect on appetite and body weight, and little tolerance is produced in the cognition enhancing effects of this dose of MPD. The present results suggest that doses of MPD which improve learning and memory are reinforcing and can predispose to addiction. A region selective decrease of 5-HT-1A receptor expression in the nucleus accumbens associated with the reinforcing effects of MPD supports the notion that 5-HT-1A receptor dependent control of the dopamine reward pathway is dysregulated. In the present study only one dose of MPD, which produced an enhancement of memory, was used for repeated administration. Future studies on the long term use of other memory enhancing as well as memory impairing doses of MPD may well show any difference in the expression of 5-HT-1A receptor associated with the reinforcing effects of MPD. Together with previous studies from our laboratory the present findings tend to suggest that targeting 5-HT-1A receptors can prevent addiction associated with the use of nootropic doses of MPD.

Acknowledgments

This work was supported by Higher Education Commission of Pakistan [Grant No. 20-3997/NRPU/R&D/HEC/14] and ICCBS, University of Karachi.

Conflict of interest

The authors declare no conflict of interest.

References

[1] D.E. Greydanus, H.D. Pratt, D.R. Patel, Attention deficit hyperactivity disorder across the lifespan: the child, adolescent, and adult, Dis. Mon. 53 (2) (2007) 70–131.
[2] J.H. Newcorn, J.M. Halperin, Attention-deficit disorders with oppositionality and aggression, Attention-deficit Disorders and Comorbidities in Children, Adolescents, and Adults, 2000, pp. 171–208.
[3] J. Fayyad, R. De Graaf, R. Kessler, et al., Cross–national prevalence and correlates of adult attention–deficit hyperactivity disorder, Br. J. Psychiatry 190 (5) (2007) 402–409.
[4] N. Imran, Attention deficit hyperactivity syndrome: an update on assessment and management, Pak. J. Med. Sci. 23 (1) (2007) 9.
[5] G. Polanczyk, M.S. De Lima, B.L. Horta, et al., The worldwide prevalence of ADHD: a systematic review and metaregression analysis, Am. J. Psychiatr. 164 (6) (2007) 942–948.
[6] S.E. McCabe, J.R. Knight, C.J. Teter, et al., Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey, Addiction 100 (1) (2005) 96–106.
[7] J. Setlik, G.R. Bond, M. Ho, Adolescent prescription ADHD medication abuse is rising along with prescriptions for these medications, Pediatrics 124 (3) (2009) 875–880.
[8] S.S. Lee, K.L. Humphreys, K. Flory, et al., Prospective association of childhood attention-deficit/hyperactivity disorder (ADHD) and substance use and abuse/dependence: a meta-analytic review, Clin. Psychol. Rev. 31 (3) (2011) 328–341.
[9] I. Dela Pena, J.C. Lee, H.L. Lee, T.S. Woo, H.C. Lee, A.R. Sohn, J.H. Cheong, Differential behavioral responses of the spontaneously hypertensive rat to methylphenidate and methamphetamine: lack of a rewarding effect of repeated methylphenidate treatment, Neurosci. Lett. 514 (2) (2012) 189–193.
[10] S.S. Somkuwar, K.M. Kantak, M.T. Bardo, L.P. Dwoskin, Adolescent methylphenidate treatment differentially alters adult impulsivity and hyperactivity in the Spontaneously Hypertensive Rat model of ADHD, Pharmacol. Biochem. Behav. 141 (2016) 66–77.
[11] I. Dela Pena, J.H. Cheong, Abuse and dependence liability analysis of methylphenidate in the spontaneously hypertensive rat model of attention-deficit/hyperactivity disorder (ADHD): what have we learned? Arch. Pharm. Res. 36 (4) (2013) 400–410.
[12] I. Dela Peña, I.J. dela Peña, J.B. de la Peña, H.J. Kim, C.Y. Shin, D.H. Han, B.N. Kim, J.H. Ryu, J.H. Cheong, Methylphenidate and atomoxetine-responsive prefrontal cortical genetic overlaps in “impulsive” SHR/NCrl and Wistar rats, Behav. Genet. 47 (5) (2017) 564–580.
[13] J. Cami, M. Farré, Drug addiction, N. Engl. J. Med. 349 (10) (2003) 975–986.
[14] D.J. Haleem, Extending therapeutic use of psychostimulants: focus on serotonin-1A receptor, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 46 (2013) 170–180.
[15] V. Engert, J.C. Pruessner, Dopaminergic and noradrenergic contributions to functionality in ADHD: the role of methylphenidate, Curr. Neuropharmacol. 6 (4) (2008) 322–328.
[16] A.F. Arnsten, S.R. Pliszka, Catecholamine influences on prefrontal cortical function: relevance to treatment of attention deficit/hyperactivity disorder and related disorders, Pharmacol. Biochem. Behav. 99 (2) (2011) 211–216.
[17] C.W. Berridge, D.M. Devilbiss, M.E. Andrzejewski, et al., Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function, Biol. Psychiatry 60 (10) (2006) 1111–1120.
[18] C.J. Loland, M. Mereu, O.M. Okunola, et al., R-modafinil (armodafinil): a unique dopamine uptake inhibitor and potential medication for psychostimulant abuse, Biol. Psychiatry 72 (5) (2012) 405–413.
[19] D.J. Haleem, S. Nawaz, T. Salman, Dopamine and serotonin metabolism associated with morphine reward and its inhibition with buspirone: a study in the rat striatum, Pharmacol. Biochem. Behav. 170 (2018) 71–78.
[20] D.J. Haleem, S. Nawaz, Inhibition of reinforcing, hyperalgesic, and motor effects of morphine by buspirone in rats, J. Pain 18 (1) (2017) 19–28.
[21] D.J. Haleem, H. Ikram, M.A. Haleem, Inhibition of apomorphine-induced conditioned place preference in rats co-injected with buspirone: relationship with serotonin and dopamine in the striatum, Brain Res. 1586 (2014) 73–82.
[22] H. Ikram, D.J. Haleem, Attenuation of apomorphine-induced sensitization by buspirone, Pharmacol. Biochem. Behav. 99 (3) (2011) 444–450.
[23] J.S. Markowitz, C.L. DeVane, S. Ramamoorthy, et al., The psychostimulant *d*-threo-(*R*, *R*)-methylphenidate binds as an agonist to the 5HT1A receptor, Die Pharm. 64 (2) (2009) 123–125.
[24] H. Whitt, N. Kharas, N. Dafny, et al., Methylphenidate modulates dorsal raphe neuronal activity: behavioral and neuronal recordings from adolescent rats, Brain Res. Bull. 128 (2017) 48–57.
[25] A.R. Preston, H. Eichenbaum, Interplay of hippocampus and prefrontal cortex in memory, Curr. Biol. 23 (17) (2013) R764–R773.
[26] J. Goodman, M.G. Packard, Memory systems and the addicted brain, Frontier Psychiatry 25 (7) (2016) 24.
[27] D.T. Chalmers, S.J. Watson, Comparative anatomical distribution of 5-HT1A receptor mRNA and 5-HT1A binding in rat brain—a combined in situ hybridisation/in vitro receptor autoradiographic study, Brain Res. 561 (1) (1991) 51–60.
[28] B.Á. Dhonnchadha, K.A. Cunningham, Serotonergic mechanisms in addiction-related memories, Behav. Brain Res. 195 (1) (2008) 39–53.
[29] D.J. Haleem, Q. Inam, M.A. Haleem, Effects of clinically relevant doses of methylphenidate on spatial memory, behavioral sensitization and open field habituation: a time related study, Behav. Brain Res. 281 (2015) 208–214.

[30] D.L. Dow-Edwards, J.C. Weedon, E. Hellmann, Methylphenidate improves performance on the radial arm maze in periadolescent rats, Neurotoxicol. Teratol. 30 (5) (2008) 419–427.
[31] M.R. Gerasimov, M. Franceschi, N.D. Volkow, A. Gifford, S.J. Gatley, D. Marsteller, P.E. Molina, S.L. Dewey, Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study, J. Pharmacol. Exp. Ther. 295 (1) (2000) 51–57.
[32] K.R. Urban, Y.C. Li, W.J. Gao, Treatment with a clinically-relevant dose of methylphenidate alters NMDA receptor composition and synaptic plasticity in the juvenile rat prefrontal cortex, Neurobiol. Learn. Mem. 101 (2013) 65–74.
[33] J. Hannestad, J.D. Gallezot, B. Planeta-Wilson, S.F. Lin, W.A. Williams, C.H. van Dyck, R.T. Malison, R.E. Carson, Y.S. Ding, Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo, Biol. Psychiatry 68 (9) (2010) 854–860.
[34] Novartis UK, Ritalin Summary of Product Characteristics, Novartis Pharmaceuticals UK Ltd., 2015 (Last updated 05 May 2015).
[35] Y. Wang, L. Lee, R. Somma, et al., In vitro dissolution and in vivo oral absorption of methylphenidate from a bimodal release formulation in healthy volunteers, Biopharm. Drug Dispos. 25 (2004) 91–98.
[36] D.J. Haleem, E. Shireen, M.A. Haleem, Somatodendritic and postsynaptic serotonin-1A receptors in the attenuation of haloperidol-induced catalepsy, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 28 (8) (2004) 1323–1329.
[37] H. Zipper, H. Brunner, J. Bernhagen, F. Vitzthum, Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications, Nucleic Acids Res. 32 (12) (2004) e103.
[38] N. Zhu, J. Weedon, D.L. Dow-Edwards, Oral methylphenidate improves spatial learning and memory in pre- and periadolescent rats, Behav. Neurosci. 121 (6) (2007) 1272.
[39] S.A. Carmack, C.L. Block, K.K. Howell, et al., Methylphenidate enhances acquisition and retention of spatial memory, Neurosci. Lett. 567 (2014) 45–50.
[40] Y.S. Chuhan, H.K. Taukulis, Impairment of single-trial memory formation by oral methylphenidate in the rat, Neurobiol. Learn. Mem. 85 (2) (2006) 125–131.
[41] D.D. Han, H.H. Gu, Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs, BMC Pharmacol. 6 (1) (2006) 6.
[42] B.J. Casey, R.M. Jones, T.A. Hare, The adolescent brain, Ann. N. Y. Acad. Sci. 1124 (1) (2008) 111–126.
[43] J.N. Giedd, Structural magnetic resonance imaging of the adolescent brain, Ann. N. Y. Acad. Sci. 1021 (1) (2004) 77–85.
[44] J. Korte, E.T. Bohlmeijer, P. Cappeliez, F. Smit, G.J. Westerhof, Life review therapy for older adults with moderate depressive symptomatology: a pragmatic randomized controlled trial, Psychol. Med. 42 (6) (2012) 1163–1173.
[45] A.F. Arnsten, Stress signalling pathways that impair prefrontal cortex structure and function, Nat. Rev. Neurosci. 10 (6) (2009) 410–422.
[46] M.A. Aguilar, M. Rodríguez-Arias, J. Miñarro, Neurobiological mechanisms of the reinstatement of drug-conditioned place preference, Brain Res. Rev. 59 (2) (2009) 253–277.
[47] J.R. Yates, J.S. Beckmann, A.C. Meyer, et al., Concurrent choice for social interaction and amphetamine using conditioned place preference in rats: effects of age and housing condition, Drug Alcohol Depend. 129 (3) (2013) 240–246.
[48] D. Zheng, S. Liu, S.C. De Vaca, et al., Effects of time of feeding on psychostimulant reward, conditioned place preference, metabolic hormone levels, and nucleus accumbens biochemical measures in food-restricted rats, Psychopharmacology 227 (2) (2013) 307–320.
[49] T.E. Wooters, M.T. Walton, M.T. Bardo, Oral methylphenidate establishes a conditioned place preference in rats, Neurosci. Lett. 487 (3) (2011) 293–296.
[50] G.F. Koob, N.D. Volkow, Neurocircuitry of addiction, Neuropsychopharmacology 35 (2009) 217–238.
[51] E.J. Nestler, S.E. Hyman, R.C. Malenka, Molecular Neuropharmacology: A Foundation for Clinical Neuroscience, McGraw-Hill Medical, 2001.
[52] M. Farhan, D.J. Haleem, Effects of chronic mild stress on apomorphine induced behavioral sensitization in different brain regions of rats in relation to serotonin change, Adv. Life Sci. 3 (1) (2015) 23–28.
[53] N. Alam, R. Najam, Effect of repeated oral therapeutic doses of methylphenidate on food intake and growth rate in rats, Pak. J. Pharm. Sci. 28 (1) (2015) 9–13.
[54] T.E. Robinson, K.C. Berridge, The psychology and neurobiology of addiction: an incentive–sensitization view, Addiction 95 (8s2) (2000) 91–117.
[55] E.K. Miller, D.J. Freedman, J.D. Wallis, The prefrontal cortex: categories, concepts and cognition, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 357 (1424) (2002) 1123–1136.
[56] Y. Yang, A. Raine, Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: a meta-analysis, Psychiatry Res. Neuroimaging 174 (2) (2009) 81–88.
[57] M. Carli, R.W. Invernizzi, Serotoninergic and dopaminergic modulation of cortico-striatal circuit in executive and attention deficits induced by NMDA receptor hypofunction in the 5-choice serial reaction time task, Front. Neural Circ. 8 (2014) 58.
[58] Y. Glikmann-Johnston, M.M. Saling, D.C. Reutens, et al., Hippocampal 5-HT1A receptor and spatial learning and memory, Front. Pharmacol. 6 (2014) (289-289).
[59] C. Davis, L. Fattore, A.S. Kaplan, et al., The suppression of appetite and food consumption by methylphenidate: the moderating effects of gender and weight status in healthy adults, Int. J. Neuropsychopharmacol. 15 (2) (2012) 181–187.
[60] J.J. Leddy, L.H. Epstein, J.L. Jaroni, et al., Influence of methylphenidate on eating in obese men, Obes. Res. 12 (2) (2004) 224–232.
[61] M.A. Stein, C.S. Sarampote, I.D. Waldman, et al., A dose-response study of OROS methylphenidate in children with attention-deficit/hyperactivity disorder, Pediatrics 112 (5) (2003) (e404-e404).
[62] V. Benard, O. Cottencin, D. Guardia, et al., The impact of discontinuing methylphenidate on weight and eating behavior, Int. J. Eat. Disord. 48 (3) (2015) 345–348.
[63] G.J. Wang, N.D. Volkow, J. Logan, et al., Brain dopamine and obesity, Lancet 357 (9253) (2001) 354–357.
[64] C. Davis, R.D. Levitan, A.S. Kaplan, et al., Food cravings, appetite, and snack-food consumption in response to a psychomotor stimulant drug: the moderating effect of "food-addiction", Front. Psychol. 5 (2014) 94–99.
[65] E. Iseri, B.G. Kilic, S. Senol, et al., Effects of methylphenidate on leptin and appetite in children with attention-deficit hyperactivity disorder: an open label trial, Methods Find. Exp. Clin. Pharmacol. 29 (1) (2007) 47–52.
[66] P.K. Thanos, L.S. Robison, J. Steier, et al., A pharmacokinetic model of oral methylphenidate in the rat and effects on behavior, Pharmacol. Biochem. Behav. 131 (2015) 143–153.
[67] A.F. Arnsten, B.M. Li, Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions, Biol. Psychiatry 57 (11) (2005) 1377–1384.
[68] T.M. Tzschentke, Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade, Addict. Biol. 12 (3–4) (2007) 227–462.
[69] T.M. Tzschentke, Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues, Prog. Neurobiol. 56 (6) (1998) 613–672.