Addiction Neuroscience xxx (xxxx) xxx

Contents lists available at ScienceDirect

Addiction Neuroscience

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

Chronic oral methylphenidate plus fluoxetine treatment in adolescent rats increases cocaine self-administration

Daniela Senior [a], Madison McCarthy [a], Rania Ahmed [a,b], Shannon Klein [a], Wen Xuan Lee [a], Michael Hadjiargyrou [c], David Komatsu [d], Heinz Steiner [e,f], Panayotis K. Thanos [a,b,*]

[a] Behavioral Neuropharmacology and Neuroimaging Laboratory (BNNL), Clinical Research Institute on Addictions, Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14051, USA
[b] Department of Psychology, University at Buffalo, Buffalo, NY, 14203, USA
[c] Department of Biological and Chemical Sciences, New York Institute of Technology, Old Westbury, NY, USA
[d] Department of Orthopedics, Stony Brook University, Stony Brook, NY, USA
[e] Stanson Toshok Center for Brain Function and Repair, Rosalind Franklin University of Medicine and Science, North Chicago, IL 60064, USA
[f] Discipline of Cellular and Molecular Pharmacology, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL 60064, USA.

ARTICLE INFO

Keywords:
Psychostimulants
Serotonin reuptake inhibitors
Addiction
Substance abuse
Reward
Behavior

ABSTRACT

Background: Depression and attention deficit hyperactivity disorder are known to be comorbid. Treatment of these commonly coexisting diseases typically involves the combined prescription of methylphenidate (MP), a psychostimulant, and fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI). MP and cocaine have similar mechanisms of action and this study examined the effects of chronic treatment of MP combined with FLX on cocaine consumption in rats.

Methods: Four groups of rats received access to drinking solutions of water (control), MP (30/60 mg/kg/day), FLX (20 mg/kg/day), or the combination of MP (30/60 mg/kg/day) plus FLX (20 mg/kg/day), during 8 h per day for one month. Following these drug treatments, rats were allowed to self-administer cocaine for 14 days.

Results: Our results showed that, during the first week of cocaine self-administration, the MP-treated rats had significantly greater numbers of active lever presses (plus 127%) and increased consumption of cocaine compared to the control rats. In contrast, during week two of cocaine self-administration, the rats treated with the MP + FLX combination showed significantly more lever presses (plus 198%) and significantly greater cocaine consumption (plus 84%) compared to the water controls.

Conclusion: Chronic oral treatment during adolescence with the combination of MP plus FLX resulted in increased cocaine use after 2 weeks of cocaine self-administration in rats. These novel findings suggest that the combined exposure to these two drugs chronically, during adolescence, may produce increased vulnerability towards cocaine abuse during young adulthood.

Introduction

Patients diagnosed with attention deficit hyperactivity disorder (ADHD) are often prescribed methylphenidate (MP), a psychostimulant also known as Ritalin. MP works by blocking the reuptake of both dopamine and norepinephrine. This causes a surge of both neurotransmitters in the synapse. Aside from its medicinal benefits, MP is often used illicitly among high school and college students to increase cognitive function or as a party drug [1,2]. Off-label use of MP is most common among 18- to 25-year-olds and recent data shows that as many as 25% of high school students engage in nonmedical use of prescription stimulants (NUPS) [3]. In fact, students that attended high schools with the greatest rates of psychostimulant therapy for ADHD had 36% increased odds of NUPS compared with students attending schools with the lowest rates [3]. ADHD is often associated with depression and anxiety. More specifically, about 12% of children have a comorbidity of anxiety. This statistic increases with patients suffering from depression as well to 16–26% [4]. The comorbidity of these mood disorders often results in co-prescription of MP and serotonin reuptake inhibitor (SSRI) antidepressants including fluoxetine (FLX) [5]. Additionally, increased

* Corresponding author to: Behavioral Neuropharmacology and Neuroimaging Laboratory (BNNL), Clinical Research Institute on Addictions, Department of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14051, USA.
E-mail address: thanos@buffalo.edu (P.K. Thanos).

https://doi.org/10.1016/j.addicn.2023.100127
Received 6 June 2023; Received in revised form 20 July 2023; Accepted 25 August 2023
Available online 26 August 2023

use of psychotropic drugs such as MP in adolescents [6] leads to increased accidental MP+SSRI co-exposure when patients on SSRIs use MP off-label. Exposure to such psychotropic medications during development is of concern, as preclinical studies demonstrated a variety of drug-induced long-term neurobehavioral changes suggestive of an increased risk for substance use disorder and other neuropsychiatric disorders later in life (e.g., [7–9]).

Combined use of MP and FLX has been shown to induce changes in body weight and behavior [10] as well as in gene regulation in the striatum that mimic effects of cocaine, and that have been previously associated with an increased risk for addictive-like behaviors [5]. Cocaine's mechanism of action is analogous to MP, blocking both dopamine and norepinephrine reuptake, hence it is important to assess the effect of combined MP+FLX treatment on cocaine consumption. The striatum is a brain region important for addiction because of its involvement in compulsive and habit forming behaviors (see, e.g., [11]). Therefore, understanding how these drug treatments will affect the striatum is crucial. A number of studies have shown that FLX potentiates effects of MP on gene regulation in the striatum ([5, 12–14]. For example, in a previous study [5], we used a chronic oral treatment regimen (in drinking water) that produces clinically relevant drug plasma levels [15] and found that MP+FLX induced changes in gene expression for the neuropeptides dynorphin and substance P, both markers for the direct output pathway of the striatum [16], which is predominantly affected by various psychostimulant treatments [11]. In this study, exposure to MP alone induced marginal increases in dynorphin and substance P mRNA levels [5]. Consumption of FLX alone did not increase gene expression; however, when FLX was combined with MP, gene expression for both neuropeptides was dramatically enhanced [5]. Moreover, although present throughout the striatum, these molecular changes were most robust in sensorimotor sectors [5], which mediate habit formation, as well as compulsive behavior (see [11]).

The above MP+FLX-induced molecular changes are more "cocaine-like" than those of MP alone in several aspects [13], which is likely based on the neurochemical impact of these drugs. MP and FLX inhibit dopamine and serotonin reuptake, respectively, the combination of both would produce greater dopamine and serotonin signaling and regulate *addiction-related genes* [11–14]. Both MP and cocaine inhibit dopamine reuptake, leading to a surge of dopamine in the synapse (e.g., [17]; for review, see [18]). However, unlike cocaine, MP does not affect serotonin reuptake [13]. Serotonin is known to contribute to the behavioral and neuronal effects of cocaine [13]. Combining serotonin reuptake inhibitors such as FLX with MP treatment thus enhances also the serotonin neurotransmission and potentiates dopamine (MP)-mediated gene regulation, mimicking cocaine effects [13]. It has been shown that repeated psychostimulant exposure facilitates subsequent cocaine self-administration in animal models [19], thus potentially increasing the addiction risk [20]. The present study thus investigated whether chronic oral MP+FLX treatment, with the same regimen that produced the previous molecular changes [5], resulted in altered cocaine intake in the cocaine self-administration model.

Materials and Methods

Subjects

3-week old male Sprague-Dawley rats (Taconic) were housed individually in temperature and humidity-controlled cages and began treatment at four weeks of age. Rats were assigned to one of four groups: water (n=13), MP (n=12), FLX (n=14), and MP+FLX (n=11). In their home cage, rats had access to water ad libitum during the first week before the drug treatment to allow proper habituation. Following habituation the rats were placed on a standard rat chow for the duration of the experiment with body weights measured daily. Food intake was measured on a weekly basis once drug treatment began. Experimental procedures followed the guidelines as described in the "Guide for the Care and Use of Laboratory Rats" in conformity with the National Academy of Science's Guide for the Care and Use of Laboratory Animals (NAS and NRC, 1996) and were approved by the State University at Buffalo Institutional Animal Care and Use Committee.

Dosing Paradigm

Oral drug treatment began at 4 weeks of age [postnatal day (PND) 28] and continued for 4 weeks. All rats were given a daily 8h limited access drinking. Methylphenidate hydrochloride (MP; Mallickrodt Pharmaceuticals, Dublin, Ireland) and fluoxetine hydrochloride (FLX; Spectrum Chemical, New Brunswick, New Jersey) were administered orally through their drinking water bottle access for eight hours a day (0900–1700). The water group had access to water for eight hours a day (0900–1700). Water access was restricted to eight hours a day for all treatment groups as previously noted in past literature [21,22]. The MP group was given access to 30 mg/kg MP for one hour (0900–1000) and 60 mg/kg for the next seven hours (1000–1700). FLX was administered at 20 mg/kg for eight hours (0900–1700) with two separate drinking bottles and concentrations (0900–1000 and 1000–1700). Bottles were prepared fresh daily by using stock MP and FLX solutions, each animals body weight and the amount of fluid consumed by each animal to produce the following doses (30/60 mg/kg or 20 mg/kg) when the bottles were consumed respectively for MP and FLX as previously described [15]. MP dosing was chosen based on previous research. In the present study, we utilized the higher dose of 30/60 mg/kg MP [15,23]. The FLX dose utilized was also based on prior research [5,24]. The MP+FLX group received a combined dose of MP (30/60 mg/kg) plus FLX (20 mg/kg) [5]. Drug exposure continued daily for four weeks (see timeline; Fig. 1).

Jugular Vein Catheterization

Following the four-week drug treatment period, each cohort underwent jugular vein catheterization (JVC) surgery in preparation for cocaine self-administration (CSA) at approximately 10 weeks old. Techniques were adopted from previous literature [25]. Briefly, rats were anesthetized using 2–3% isoflurane. Throughout the surgery, breathing and the general health of the rats were monitored. Once the rat was anesthetized and pedal reflexes were checked, the surgery site was properly sterilized. A 3 cm horizontal incision was made in the upper lateral portion of the rats' chest. Absorbable sutures were placed to anchor the catheter to the vein. Blunt dissection was used to tunnel to the dorsal portion of rat, where the port was pulled through. Once finalized, both the ventral and dorsal incisions were sterilely closed with absorbable sutures. JVC surgeries were followed by three consecutive days of post-operative care. During the post-operative period (3–7 days depending on the animals recovery), rats received both Rimadyl (5 mg/kg) and Baytril (5 mg/kg) via subcutaneous injections once a day for

Fig. 1. Project Timeline During weeks 0 through 28, daily oral drug treatment was administered for 8 h daily (0900–1700). After 4 weeks of drug treatment, Food administration training began (days 28–31). Utilizing the self-administration paradigm, food training continued until 95% efficiency and 4 days of training were completed. Once proficient, the animals were operated on and received the jugular vein catheter. After surgery and post-operative care (days 31–35), the animals began cocaine self-administration (days 35–49).

a minimum of three days, along with topical neomycin application to the incisions. Body weights and diet were carefully monitored to ensure the health and safety of the animals. In addition, catheters were flushed twice daily with heparin (30 units/mL), baytril (22.7 mg/mL) and saline, to maintain cannula patency.

Cocaine Self-Administration

Cocaine self-administration (CSA) was performed following completion of drug treatment (see timeline; Fig. 1). All procedures were administered in standard operant chambers containing two retractable levers, a cue light, a house light, and tone generator [26]. Prior to the start of cocaine administration all animals underwent three consecutive days of 2 h food training in the operant chambers as previously described [26]. Once the rats were trained to lever press for food and reached a criteria [95% of total responses were on the active (food) lever, and less than 5% from inactive lever for a minimum three days] they began the cocaine self-administration experiment (CSA) which was conducted for 14 days. CSA sessions lasted for 2 h each day, and cocaine was administered on a FR1 schedule with a 30 s timeout. Active lever presses were followed by an infusion of (0.3 mg/kg) cocaine. During the timeout, cocaine was not dispensed, however, the lever presses were recorded. Inactive lever presses served as an activity control. Pressing the inactive lever resulted in no cocaine infusions.

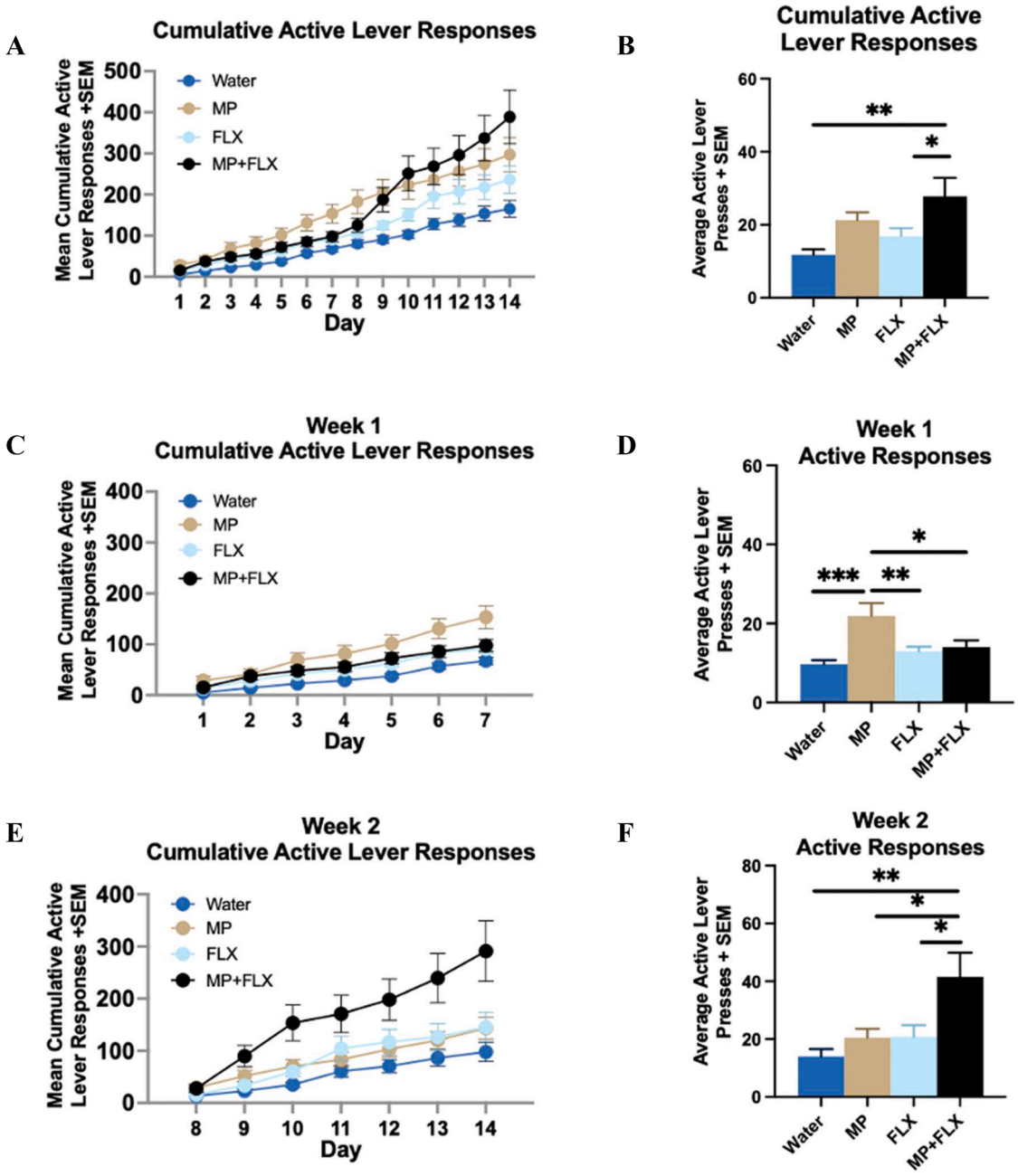

Fig. 2. Active lever responses during 14 days of cocaine self-administration. (A) Mean (+/- SEM) number of cumulative active lever responses over 14 days, and (B) average number per day (mean +/- SEM) of active lever presses for the 14 days, in rats that had previously received water, methylphenidate (MP), fluoxetine (FLX) or methylphenidate plus fluoxetine (MP+FLX) in their drinking water for 28 days. Mean (+/- SEM) number of cumulative active lever responses during week 1 (C) and week 2 (E), and average number per day (mean +/- SEM) of active lever presses for week 1 (D) and week 2 (F) are also shown. *p<.05; **p<.01; ***p<.001.

Statistics

Results are presented as cumulative daily cocaine infusions, active lever presses and inactive lever presses over all 14 days and for weeks 1 and 2, separately. A one-way ANOVA was conducted between the 4 different treatment groups (water, MP, FLX or MP+FLX) for active lever responses, inactive lever responses, as well as cocaine infusions. Significant ANOVA results were followed up with Tukey's HSD post-hoc analysis to describe differences between individual groups. All statistical analyses were performed using Prism software (GraphPad; San Diego, CA, US) with statistical significance set as a = 0.05.

Results

Lever Responses

A main effect of drug treatment was found for active lever responses accumulated over the 14 days [F(3,94)=5.110; p < .01; Fig. 2B]. Post-hoc analysis using the Tukey HSD test showed that in weeks 1 and 2 cumulatively, MP+FLX-treated rats had 135% more active lever responses compared to water-treated controls ($p < .01$; Fig. 2B). There were also main effects of drug treatment for active lever responses in week 1 [$F(3,45) = 6.984$; $p < .001$; Fig. 2D] and week 2 [$F(3,45) = 5.811$; $p < .01$; Fig. 2F], separately. Post-hoc comparisons using the Tukey HSD test showed that in week 1, MP-treated rats had 127% more active lever responses compared to water-treated rats ($p < .001$; Fig. 2D). In week 2, MP+FLX-treated rats had 198% more active lever responses compared to water-treated rats ($p < .01$; Fig. 2F). MP+FLX-treated rats had also significantly more active lever responses than MP-treated ($p < .05$) and FLX-treated rats ($p < .05$) (Fig. 2F). No treatment effects were found for inactive lever presses for either week 1 [$F(3,45) = 0.3237$; $p > .05$; Fig. 3B] or week 2 [$F(3,45) = 2.599$; $p > .05$; Fig. 3C].

Cocaine Infusions

A main effect of drug treatment was also found for cocaine consumption (infusions) across the 14 days [F(3,94)=5.331; p < .01] (Fig. 4B). Post-hoc analysis using the Tukey HSD test revealed that in weeks 1 and 2 cumulatively, MP-treated rats had 57% more cocaine infusions compared to the water control group ($p < .05$; Fig. 4B). Additionally, MP+FLX-treated rats had 66% more infusions compared to the water control ($p < .05$; Fig. 4B) and more infusions compared to the FLX-treated rats ($p < .05$; Fig. 4B). Main effects of treatment were again found for cocaine infusions within both week 1 [$F(3,45) = 3.537$; $p < .05$; Fig. 4D] and week 2 [$F(3,45) = 3.447$; $p < .05$; Fig. 4F]. Post-hoc comparisons using Tukey's HSD test showed that during week 1, MP-treated rats had 64% more cocaine infusions compared to the water control group ($p < .05$; Fig. 4D). In contrast, during week 2, MP+FLX-treated rats showed 84% more infusions compared to the control ($p < .05$; Fig. 4F)

Discussion

Our most important findings are summarized as follows. Four weeks of oral treatment with MP, FLX or the combination of MP+FLX (in their drinking water) in adolescent rats produced significant changes in subsequent cocaine self-administration behavior. During week 1 of acquisition of cocaine self-administration, rats pre-treated with MP alone displayed significantly more active lever presses than any of the other 3 groups and more cocaine infusions than the water control group. During week 2 cocaine self-administration was more stable and rats pre-treated with MP+FLX showed significantly more active lever presses and cocaine infusions than control rats. Therefore, while MP treatment alone facilitated the early acquisition of cocaine taking behavior, the combined MP+FLX treatment greatly enhanced cocaine seeking behavior after that.

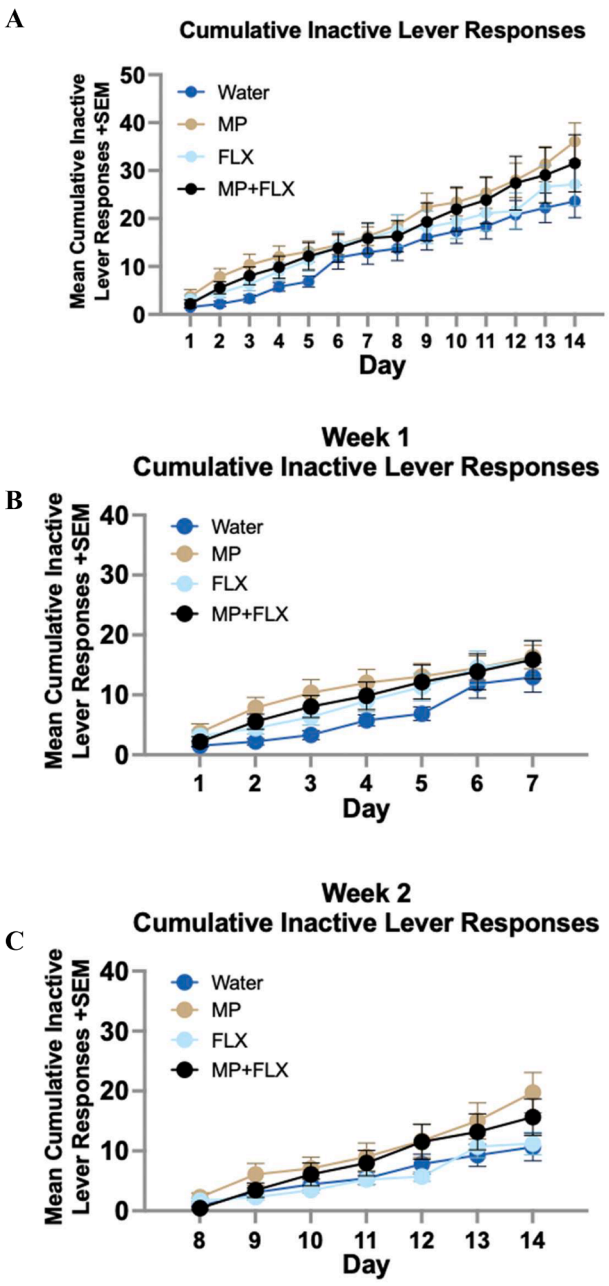

Fig. 3. Inactive lever responses during 14 days of cocaine self-administration. Mean (+/- SEM) number of cumulative inactive lever responses over 14 days (A), during week 1 (B) and week 2 (C) are given for rats that had previously received water, methylphenidate (MP), fluoxetine (FLX) or methylphenidate plus fluoxetine (MP+FLX) in their drinking water for 28 days.

In the present study, MP and FLX were administered orally in adolescent male rats. Future studies need to extend these results using females. The oral doses of MP corresponded to clinically similar pharmacokinetic levels that were determined based on previous studies in rats [15,23,25]. The FLX dose was also based on prior research [5,24]. Compared to the water control group, chronic oral treatment with MP, FLX, or the combination (MP+FLX) in these rats resulted in an overall lower body weight during the 4 weeks of treatment. These findings were in agreement with those of prior studies following chronic MP administration in rats [27], an effect also observed in clinical studies [28,29]. Similarly, a decrease in body weight was observed with FLX treatment [24,30,31,10]. The decrease in body weight for the combined MP+FLX group was also in agreement with our recent study [32]. The weight loss

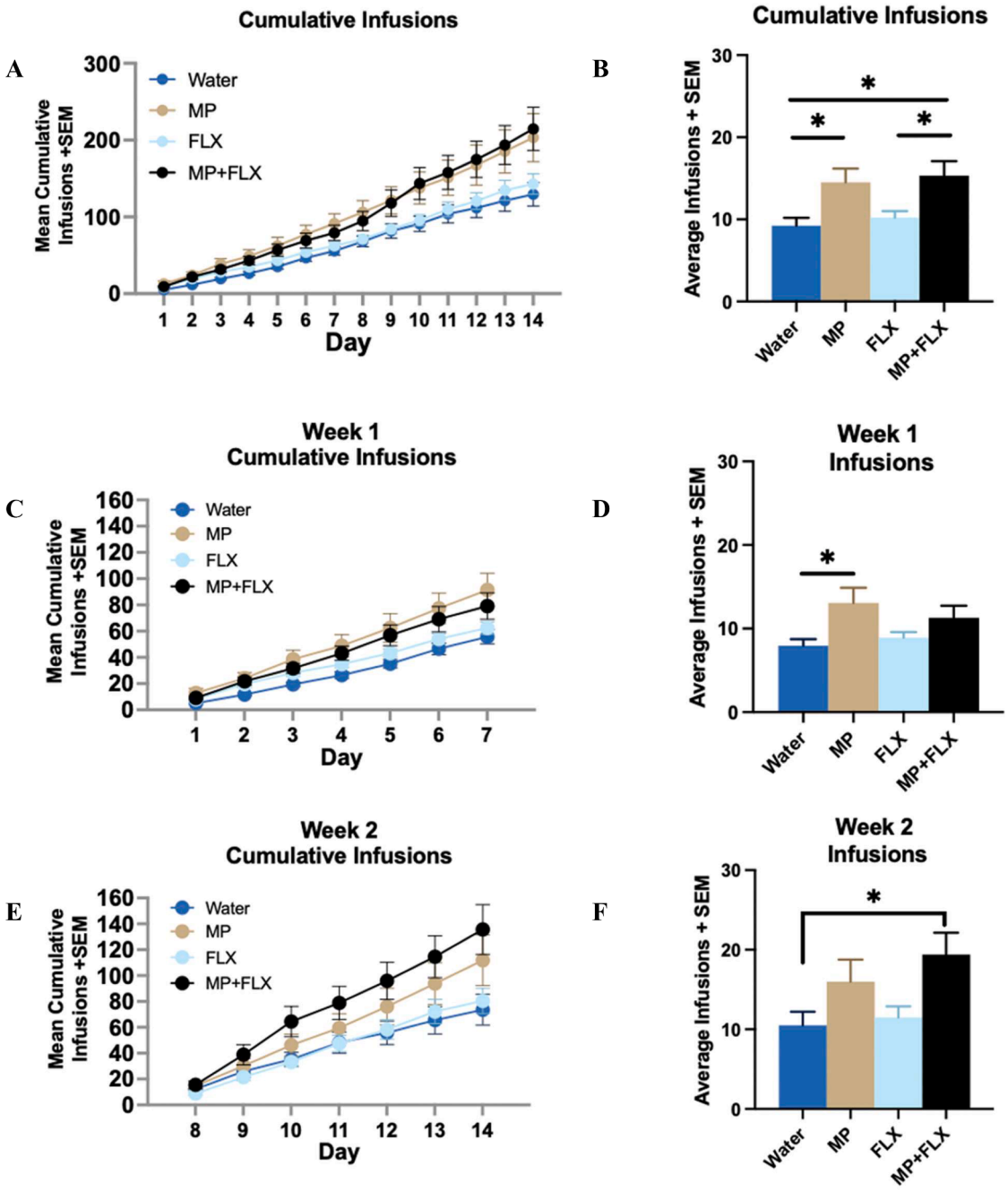

Fig. 4. Cocaine infusions during 14 days of cocaine self-administration. (A) Mean (+/- SEM) number of cumulative infusions over 14 days, and (B) average number per day (mean +/- SEM) of infusions for the 14 days, in rats that had previously received water, methylphenidate (MP), fluoxetine (FLX) or methylphenidate plus fluoxetine (MP+FLX) in their drinking water for 28 days. Mean (+/- SEM) number of cumulative infusions during week 1 (C) and week 2 (E), and average number per day (mean +/- SEM) of infusions for week 1 (D) and week 2 (F) are also shown. *p<.05.

in the MP+FLX combined treated rats underlines the importance of weight monitoring when these drugs are administered together, specifically for subjects with prior low body mass index (BMI).

We examined the active lever pressing behavior across experimental groups. Overall, there were significant differences in active lever pressing after MP and MP+FLX treatments. While MP only-treated rats showed significantly enhanced active lever pressing compared to the other groups (water control, FLX, and MP+FLX) in week 1, MP+FLX-treated rats displayed significantly more active lever presses than all the other experimental groups in week 2. Inactive lever presses were monitored as a control for general activity. No significant differences were observed between these groups. These results show that the changed lever pressing behavior was specific to the cocaine-delivering active lever and not related to generalized increased activity. Locomotor activity was not recorded in this study. There were no significant differences in inactive lever presses (Fig. 3). If there were locomotor differences between the groups, one would expect significant differences in the inactive lever presses as well. Future research into locomotor activity during cocaine self-administration is needed. The cocaine intake (number of infusions) paralleled the differences in active lever pressing, with significantly more cocaine infusions in MP-treated animals than water controls in week 1 and even greater cocaine consumption (infusions) in the MP+FLX-treated animals (compared to controls in week 2).

The increased lever pressing for cocaine and cocaine intake after MP pre-treatment found in week 1 agreed with previous findings in studies using various MP doses for repeated treatment at different developmental stages. Thus, facilitated acquisition of cocaine self-administration was demonstrated subsequent to MP treatment in pre-weanling (MP 2 mg/kg, i.p., PND 11–20; [33], adolescent (2 mg/kg, i.p., PND 36–42; [34]), and adult rats (20 mg/kg, i.p.; [35]). These earlier findings were obtained with intermittent i.p. injections of MP, which are of course not as clinically similar and produce a very different pharmacokinetic profile (compared to oral dosing) with higher plasma peak levels, and may thus be more relevant for intermittent MP abuse. Our present findings are to our knowledge the first to show enhanced cocaine intake following oral administration of MP with clinically relevant MP plasma levels [15]. Other studies have been reported with lower oral doses of MP. For example, one study treated juvenile rats (PND 20) with MP (2 mg/kg/day, orally) for three weeks and found no effect on cocaine self-administration 6 weeks later [36]. In another study, rats chronically administered with a lower dose of oral MP (2 mg/kg/day) starting at PND 28 showed significant reductions in dopamine D2 receptor levels which is associated with an increased propensity for self-administration of drugs both in laboratory animals and in humans [25] and that this risk may be mediated by the duration of treatment. These findings indicate that the treatment regimen (e.g., doses, duration) and testing variables are important for the outcome.

The present study is the first to investigate the effects of combined oral MP+FLX treatment on cocaine self-administration in a non-ADHD model. Future studies should be done to observe the effects of combined treatment on cocaine self-administration in an ADHD model. FLX alone has no significant effect on CSA and in contrast to our MP-only pretreatment, our findings in the combination treatment group (MP+FLX) demonstrated significantly greater cocaine consumption compared to the controls, during week 2. MP inhibits dopamine reuptake by blocking the dopamine transporter [37]. Adding the SSRI, FLX to MP will increase extracellular serotonin levels in addition to the elevated dopamine levels, with the combination thus mimicking more closely the neurochemical effects of cocaine, which blocks dopamine and serotonin reuptake (for review, see [18]). A series of studies demonstrated that FLX potentiates MP-induced gene regulation in the forebrain [13], presumably via stimulation of the dopamine neurotransmission by serotonin action [13].

Enhanced gene regulation in the striatum by MP+FLX vs. MP includes greater molecular responses to cocaine subsequent to repeated MP+FLX exposure [38,39], as well as elevated expression of serotonin receptors and neuropeptides, notably dynorphin [14], with some of these molecular adaptations lasting for at least 2 weeks after the repeated treatment [38]. While the above studies used repeated i.p. drug administration of relatively high doses, mimicking abuse doses [13], our more recent study [5] investigated the effects of more clinically relevant administration of MP and FLX, using the same oral treatment regimen as employed in the present study. Our results demonstrated that potentiated gene regulation also occurs with this oral MP+FLX treatment. These effects included a very robust upregulation of dynorphin expression in most striatal areas, including the nucleus accumbens [5].

Dynorphin is an opioid peptide that is notably important during addiction, as it regulates dopamine input to the striatum and other processes (for reviews, see [11,40]). This opioid peptide has been shown to be upregulated by chronic exposure to a variety of psychostimulants, in rodents but also in primates and human cocaine addicts [11,13]. Our present findings show that our repeated oral MP+FLX treatment regimen, which induces upregulated dynorphin expression throughout the striatum and nucleus accumbens [41], produces enhanced cocaine consumption in the self-administration model.

The present findings are very important in the context of several theories on addiction including the Gateway theory, which suggests that exposure to certain drugs at an early age can lead to abuse of other harder drugs later in life [42]. This is somewhat of a nuanced area of research because addiction and motivations for drug use can be influenced by many other factors including social, environmental, and genetic. See previous research on strengths and weaknesses of this theory: [43,44]. Research has been shown that MP can be a “gateway” drug because as a psychostimulant it engages similar mechanisms as “harder” stimulants such as cocaine [45]. In addition, our results show that combining MP with an SSRI like FLX produces greater molecular changes, mimicking cocaine effects [13]. The gateway theory also helps explain why individuals with ADHD have an accelerated and increased risk of future illicit drug abuse during their adulthood [46]. Studies have found that the earlier the exposure to MP occurs, the more likely this will lead to neurobehavioral consequences such as reduction of sensitivity to the drug and possible difficulties with self-control, leading to addictive behaviors [9].

Reward Deficiency Syndrome (RDS) involves a hypodopaminergic state of the brain caused by both genetic and epigenetic factors [47,48]. Previous research on RDS has described that those diagnosed with ADHD have hypodopaminergic state and share many phenotypes and molecular attributes with patients with substance use disorder, i.e., deficits in dopamine receptors and other changes [49]. Therefore, the chronic use of MP may help counter aspects of the hypodopaminergic state [50]. However, combining MP with SSRIs appears to induce greater or other molecular changes that may become maladaptive, for example, by increasing the risk of substance use disorder or other neuropsychiatric disorders, [22,51].

In summary, this study demonstrated that chronic (1 month) oral treatment with MP or MP+FLX in adolescent rats increased cocaine self-administration behavior. Specifically, while the MP-treated animals displayed faster acquisition (week 1), the MP+FLX-treated rats showed a 198% increase in cocaine lever presses, as well as an 84% increase in cocaine consumption in comparison to controls.

Conclusion

The current study investigated the effects of chronic oral treatment with MP, FLX or the combination of MP and FLX in adolescent rats on cocaine self-administration. The results showed that the MP+FLX combination led to an 84% increase in cocaine consumption by the end of the second week. These findings support the notion that combining psychostimulants with an SSRI drug may increase the risk for future cocaine abuse. Future clinical research will have to investigate whether these preclinical findings translate to treatments with the combination of MP and FLX in patients.

Author Contributions

Conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: DS, MM, RA, SK, WXL, PKT; Drafting the work or revising it critically for important intellectual content: DS, MM, RA, PKT; Final approval of the version to be published: PKT; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: MH, DK, HS, PKT.

Funding

This work was supported by grants from NICHD (1R01HD070888-01A1) and the Research Foundation of NY (1115107) to PKT, and NIDA (R01DA046794) to HS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank Alexandra Carvalho for animal care assistance.

References

[1] C.J. Teter, et al., Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration, Pharmacotherapy 26 (10) (2006) 1501–1510.
[2] S.N. Visser, et al., Trends in the parent-report of health care provider-diagnosed and medicated attention-deficit/hyperactivity disorder: United States, 2003-2011, J. Am. Acad. Child Adolesc. Psychiatry 53 (1) (2014) 34–46, e2.
[3] S.E. McCabe, et al., Prescription stimulant medical and nonmedical use among US secondary school students, 2005 to 2020, JAMA Netw. Open 6 (4) (2023), e238707.
[4] A. Chronis-Tuscano, et al., Very early predictors of adolescent depression and suicide attempts in children with attention-deficit/hyperactivity disorder, Arch. Gen. Psychiatry 67 (10) (2010) 1044–1051.
[5] C. Moon, et al., Fluoxetine potentiates oral methylphenidate-induced gene regulation in the rat striatum, Mol. Neurobiol. 58 (10) (2021) 4856–4870.
[6] I. Hartz, et al., Psychotropic drug use among 0–17 year olds during 2004–2014: a nationwide prescription database study, BMC Psychiatry 16 (1) (2016) 12.
[7] Jr W.A. Carlezon, C. Konradi, Understanding the neurobiological consequences of early exposure to psychotropic drugs: linking behavior with molecules, Neuropharmacology 1 (0 1) (2004) 47–60, 47 Suppl.
[8] S.D. Mague, S.L. Andersen, W.A. Carlezon, Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats, Biol. Psychiatry 57 (2) (2005) 120–125.
[9] E.M. Marco, et al., Neurobehavioral adaptations to methylphenidate: the issue of early adolescent exposure, Neurosci. Biobehav. Rev. 35 (8) (2011) 1722–1739.
[10] A. Serretti, L. Mandelli, Antidepressants and body weight: a comprehensive review and meta-analysis, J. Clin. Psychiatry 71 (10) (2010) 1259–1272.
[11] H. Steiner, V. Van Waes, Addiction-related gene regulation: risks of exposure to cognitive enhancers vs. other psychostimulants, Prog. Neurobiol. 100 (2013) 60–80.
[12] H. Steiner, V. Van Waes, M. Marinelli, Fluoxetine potentiates methylphenidate-induced gene regulation in addiction-related brain regions: concerns for use of cognitive enhancers? Biol. Psychiatry 67 (6) (2010) 592–594.
[13] V. VanWaes, H. Steiner, Fluoxetine and other SSRI antidepressants potentiate addiction-related gene regulation by psychostimulant medications. Fluoxetine: Pharmacology, Mechanisms of Action and Potential Side Effects, Nova Science Publishers, New York, 2015, pp. 207–225.
[14] V. Van Waes, et al., Fluoxetine potentiation of methylphenidate-induced gene regulation in striatal output pathways: potential role for 5-HT1B receptor, Neuropharmacology 89 (2015) 77–86.
[15] P.K. Thanos, et al., A pharmacokinetic model of oral methylphenidate in the rat and effects on behavior, Pharmacol. Biochem. Behav. 131 (2015) 143–153.
[16] H. Steiner, C.R. Gerfen, Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior, Exp. Brain Res. 123 (1-2) (1998) 60–76.
[17] E.S. Calipari, S.R. Jones, Sensitized nucleus accumbens dopamine terminal responses to methylphenidate and dopamine transporter releasers after intermittent-access self-administration, Neuropharmacology 82 (2014) 1–10.
[18] M. Yano, H. Steiner, Methylphenidate and cocaine: the same effects on gene regulation? Trends Pharmacol. Sci. 28 (11) (2007) 588–596.
[19] P. Vezina, Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs, Neurosci. Biobehav. Rev. 27 (8) (2004) 827–839.
[20] M. Krank, et al., Structural, concurrent, and predictive validity of the Substance Use Risk Profile Scale in early adolescence, Addict. Behav. 36 (1) (2011) 37–46.
[21] D. Senior, et al., Behavioral, neurochemical and developmental effects of chronic oral methylphenidate: a review, J Pers Med 13 (4) (2023).
[22] P.K. Thanos, et al., Combined chronic oral methylphenidate and fluoxetine treatment during adolescence: effects on behavior, Curr. Pharm. Biotechnol. 24 (10) (2023) 1307–1314.
[23] C. Martin, et al., Recovery from behavior and developmental effects of chronic oral methylphenidate following an abstinence period, Pharmacol. Biochem. Behav. 172 (2018) 22–32.
[24] A. Aggarwal, et al., Selective serotonin re-uptake inhibitors (SSRIS) induced weight changes: a dose and duration dependent study on albino rats, J. Clin. Diagn. Res. JCDR 10 (3) (2016). AF01-AF3.
[25] P.K. Thanos, et al., Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents, Pharmacol. Biochem. Behav. 87 (4) (2007) 426–433.
[26] L.A. Knackstedt, et al., Extinction training after cocaine self-administration induces glutamatergic plasticity to inhibit cocaine seeking, J. Neurosci. 30 (23) (2010) 7984–7992.
[27] E. Carias, et al., Weekday-only chronic oral methylphenidate self-administration in male rats: Reversibility of the behavioral and physiological effects, Behav. Brain Res. 356 (2019) 189–196.
[28] M. Schertz, et al., Predictors of weight loss in children with attention deficit hyperactivity disorder treated with stimulant medication, Pediatrics 98 (4) (1996) 763–769. Pt 1.
[29] A. Poulton, et al., Weight loss on stimulant medication: how does it affect body composition and bone metabolism? - A prospective longitudinal study, Int. J. Pediatr. Endocrinol. 2012 (1) (2012), 30-30.
[30] M.R. Hodges, et al., Fluoxetine augments ventilatory CO2 sensitivity in brown norway but not sprague dawley rats, Respir. Physiol. Neurobiol. 186 (2) (2013) 221–228.
[31] J.P. Domecq, et al., Clinical review: Drugs commonly associated with weight change: a systematic review and meta-analysis, J. Clin. Endocrinol. Metab. 100 (2) (2015) 363–370.
[32] P.K. Thanos, et al., Combined Chronic Oral Methylphenidate and Fluoxetine Treatment During Adolescence: Effects on Behavior, Current Pharmaceutical Technology; Bentham Science, 2022.
[33] C.A. Crawford, et al., Early methylphenidate exposure enhances cocaine self-administration but not cocaine-induced conditioned place preference in young adult rats, Psychopharmacology 213 (1) (2011) 43–52 (Berl.).
[34] C.L. Brandon, et al., Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats, Neuropsychopharmacology 25 (5) (2001) 651–661.
[35] S. Schenk, S. Izenwasser, Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine, Pharmacol. Biochem. Behav. 72 (3) (2002) 651–657.
[36] N. Freund, et al., Juvenile exposure to methylphenidate and guanfacine in rats: effects on early delay discounting and later cocaine-taking behavior, Psychopharmacology 236 (2) (2019) 685–698 (Berl.).
[37] N.D. Volkow, et al., Relationship between blockade of dopamine transporters by oral methylphenidate and the increases in extracellular dopamine: Therapeutic implications, Synapse 43 (3) (2002) 181–187.
[38] J.A. Beverley, et al., Potentiated gene regulation by methylphenidate plus fluoxetine treatment: Long-term gene blunting (Zif268, Homer1a) and behavioral correlates, Basal Ganglia 4 (3) (2014) 109–116.
[39] V. Van Waes, et al., Selective serotonin re-uptake inhibitors potentiate gene blunting induced by repeated methylphenidate treatment: Zif268 versus Homer1a, Addict. Biol. 19 (6) (2014) 986–995.
[40] T.S. Shippenberg, A. Zapata, V.I. Chefer, Dynorphin and the pathophysiology of drug addiction, Pharmacol. Ther. 116 (2) (2007) 306–321.
[41] C. Moon, et al., Fluoxetine potentiates oral methylphenidate-induced gene regulation in the rat striatum, Mol. Neurobiol. 58 (10) (2021) 4856–4870.
[42] M.T. Lynskey, A. Agrawal, Denise Kandel's classic work on the gateway sequence of drug acquisition: Kandel's gateway theory of drug use, Addiction 113 (10) (2018) 1927–1932.
[43] S. Nkansah-Amankra, M. Minelli, Gateway hypothesis" and early drug use: Additional findings from tracking a population-based sample of adolescents to adulthood, Prev. Med. Rep. 4 (2016) 134–141.
[44] L. Degenhardt, et al., Evaluating the drug use "gateway" theory using cross-national data: consistency and associations of the order of initiation of drug use among participants in the WHO world mental health surveys, Drug Alcohol Depend. 108 (1-2) (2010) 84–97.
[45] K. Fredriksen, The gateway Theory and Adolescent Substance Use, ProQuest Dissertations Publishing, 2006.
[46] E.M. Dunne, et al., ADHD as a risk factor for early onset and heightened adult problem severity of illicit substance use: an accelerated gateway model, Addict. Behav. 39 (12) (2014) 1755–1758.
[47] M.S. Gold, et al., Neurological correlates of brain reward circuitry linked to opioid use disorder (OUD): Do homo sapiens acquire or have a reward deficiency syndrome? J. Neurol. Sci. 418 (2020), 117137-117137.
[48] K. Blum, P.K. Thanos, M.S. Gold, Dopamine and glucose, obesity, and reward deficiency syndrome, Front. Psychol. 5 (2014) 1–11.
[49] K. Blum, et al., Attention-deficit-hyperactivity disorder and reward deficiency syndrome, Neuropsychiatr. Dis. Treat. 4 (5) (2008) 893–918.
[50] L.S. Robison, et al., Chronic oral methylphenidate treatment reversibly increases striatal dopamine transporter and dopamine type 1 receptor binding in rats, J. Neural Transm. 124 (5) (2017) 655–667.
[51] B.L. Warren, et al., Juvenile administration of concomitant methylphenidate and fluoxetine alters behavioral reactivity to reward- and mood-related stimuli and disrupts ventral tegmental area gene expression in adulthood, J. Neurosci. 31 (28) (2011) 10347–10358.