

Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents

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Abstract

Background: Methylphenidate (MP) and amphetamine, which are the mainstay for the treatment of ADHD, have raised concerns because of their reinforcing effects and the fear that their chronic use during childhood or adolescence could induce changes in the brain that could facilitate drug abuse in adulthood.

Methods: Here we measured the effects of chronic treatment (8 months) with oral MP (1 or 2 mg/kg), which was initiated in periadolescent rats (postnatal day 30). Following this treatment, rats were tested on cocaine self-administration. In addition at 2 and 8 months of treatment we measured dopamine D2 receptor (D2R) availability in the striatum using [¹¹C]raclopride microPET (μPET) imaging.

Results: Animals treated for 8 months with 2 mg/kg of MP showed significantly reduced rates of cocaine self-administration at adulthood than vehicle treated rats. D2R availability in the striatum was significantly lower in rats after 2 months of treatment with MP (1 and 2 mg/kg) but significantly higher after 8 months of MP treatment than in the vehicle treated rats. In vehicle treated rats D2R availability decreased with age whereas it increased in rats treated with MP. Because low D2R levels in the striatum are associated with a propensity for self-administration of drugs both in laboratory animals and in humans, this effect could underlie the lower rates of cocaine self-administration observed in the rats given 8 months of treatment with MP.

Conclusions: Eight month treatment with oral MP beginning in adolescence decreased cocaine-self administration (1 mg/kg) during adulthood which could reflect the increases in D2R availability observed at this life stage since D2R increases are associated with reduced propensity for cocaine self-administration. In contrast, two month treatment with MP started also at adolescence decreased D2R availability, which could raise concern that at this life stage short treatments could possibly increase vulnerability to drug abuse during adulthood. These findings indicate that MP effects on D2R expression in the striatum are sensitive not only to length of treatment but also to the developmental stage at which treatment is given. Future studies evaluating the effects of different lengths of treatment on drug self-administration are required to assess optimal duration of treatment regimes to minimize adverse effects on the propensity for drug self-administration.

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1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is the most commonly diagnosed and treated psychiatric disorder of childhood; its prevalence is estimated to be 5–10% of the general population in the USA (Swanson et al., 1998). Methylphenidate (MP), commonly known by brand name as Ritalin® or Adderall® (which is a mix of dextro- and racemic amphetamine

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salts), and amphetamine (AMP) are the most frequently used treatments for ADHD (Greenhill et al., 2002). Over the past decade, the prescriptions for these stimulant medications have increased from less than 2 million in 1991 to over 22 million in 2001, and now it is estimated that up to 6% of school-aged children are treated with these drugs.

MP and AMP increase extracellular dopamine (DA) in the brain, as do cocaine and methamphetamine, the most commonly abused stimulant drugs. MP (like cocaine) increases DA by blocking DA transporters (DAT) (Volkow et al., 1995) and AMP (like methamphetamine) increases DA by releasing DA from the terminals (Jones et al., 1998). Both increase DA in the nucleus accumbens (NAc), which is thought to underlie the reinforcing effects of drugs of abuse (Di Chiara and Imperato, 1988). Indeed, MP and AMP are self-administered by animals (Bergman et al., 1989; Johanson and Schuster, 1975) and sometimes used recreationally by humans (Foley et al., 2000; Kollins et al., 2001). This has raised concerns about the abuse liability of MP and AMP and the potential that their chronic use during adolescence may induce long-term changes that facilitate drug abuse in adulthood. One clinical study has shown diminished rates of substance abuse in ADHD children and adolescents treated with stimulant medications compared with controls (Wilens et al., 2003). Short-term (5–7 days) ip methylphenidate treatment in adolescent rats was shown to enhance reactivity to cocaine and vulnerability to cocaine self-administration (Brandon et al., 2001). So while it is recognized that ADHD per se constitutes a risk for substance abuse, the role of stimulant treatment during adolescence on drug abuse during adulthood is still not properly understood. Chronic oral MP treatment in adolescent rats followed by access to intravenous cocaine self-administration as adults remains to be examined.

Animal studies investigating the effects of chronic exposure to MP during the developmental periods corresponding to human childhood and adolescence have shown changes in the function of brain DA cells, as well as changes in behavior (including enhanced sensitivity to stressful stimuli, decreased response to natural reinforcers, and decreased threshold for helplessness) and altered responses to the reinforcing properties of cocaine in adulthood (Bolanos et al., 2003; Brandon et al., 2001, 2003; Carlezon et al., 2003). The latter effect depended on when the initial exposure occurred: when administered to young rats at an age corresponding to childhood in humans, MP exposure decreased the reinforcing properties of cocaine (Carlezon et al., 2003), but when administered to rats at an age roughly equivalent to adolescence in humans, it increased them (Brandon et al., 2001).

MP has also been shown to produce changes in gene expression consistent with the effects of other psychostimulants. In particular, striatal dose-dependent increases in the expression of immediate-early genes (*c-fos* and *zif 268*; which encode transcription factors) have been shown to be induced by both acute and chronic MP (Brandon and Steiner, 2003; Yano and Steiner, 2005a,b), as well as synaptic plasticity factor Homer 1A. Daily MP (10 mg/kg, ip) treatment for a week led to increases in *c-fos* and *zif268* gene expression as well as dynorphin expression in the striatum of adolescent rats, indicating that short term expo-

sure to MP had some significant epigenetic effects which could lead to a cascade of molecular and cellular changes associated with DA receptor overstimulation.

Here we assess the effects of chronic oral MP treatment in the adolescent rat. We gave MP orally since this is the route of administration used therapeutically for ADHD and at comparable doses (1 mg/kg and 2 mg/kg) that have been shown to be comparable to those used clinically (Gerasimov et al., 2000). We measured striatal D2R availability in-vivo using [¹¹C]raclopride μ PET imaging; since low D2R levels are associated with a vulnerability to the reinforcing effects of drugs of abuse in laboratory animals and in humans (Volkow, 1997; Volkow et al., 1999a,b; Volkow et al., 2002) whereas high levels appear to be protective against drug self-administration. Also, chronic treatment with cocaine, which is pharmacologically similar to MP (Volkow et al., 1999a,b) has been previously shown to induce long lasting decreases in D2R in non human primates, (Nader et al., 2002) and cocaine abusers have long lasting reductions in D2R (Volkow et al., 1997). We also measured the effects of chronic MP treatment on subsequent cocaine self-administration behavior. Thus, this study was conducted to measure the effects of chronic MP exposure beginning in adolescence on: 1) striatal D2R availability and 2) cocaine self-administration behavior. We hypothesized that chronic MP exposure would result in a lower D2R availability and an increase in cocaine self-administration.

2. Materials and methods

2.1. Animals

Male 4-week old Sprague–Dawley rats (Taconic Farms) were individually housed in a temperature-humidity controlled environment under a 12-hour light/12-hour dark cycle. Animals were randomly assigned into 3 groups ($N=6$ /group): 2 mg/kg MP, 1 mg/kg MP, and water (control) and were given access to Purina Rat Chow and water ad libitum. All experiments were conducted in conformity with the National Academy of Sciences Guide for Care and Use of Laboratory Animals (National Academy of Sciences NRC et al., 1996) and Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols. Drinking bottles were filled with the appropriate solution, with a volume equal to the mean volume consumed by the control animals. All solutions were prepared fresh daily for corresponding doses of MP. Body weight was recorded daily throughout the experiment. All MP-treated animals were given volumes of the MP in water solution based on the volume consumed by the control animals of similar age and weight that drank a water solution. MP solutions were prepared fresh daily according to these volumes to achieve the desired concentrations. We did not see any indication of taste-aversion given that all animals drank the entire solution daily.

2.2. Drugs

MP hydrochloride (Sigma, St. Louis, MO) was dissolved in distilled water to produce concentrations of 2 mg/kg and 1 mg/kg.

Cocaine hydrochloride (1 mg/kg; Sigma, St. Louis, MO) was dissolved in saline (0.9% NaCl).

2.3. Procedures

2.3.1. Experiment 1. D2R availability

2.3.1.1. D2R μ PET imaging. μ PET assessment of D2R was performed using a μ PET R4 Scanner (CTI Concorde, Knoxville, TN). Each animal was anesthetized and injected intravenously with [11 C]raclopride (a D2R-specific ligand) and scanned two times: a) after 2 months of treatment (3 months of age) and b) after 8 months of treatment (9 months of age). Raclopride was purchased from Sigma and [11 C]raclopride was synthesized in the Brookhaven National Laboratory Cyclotron as previously described (Fowler and Volkow, 1998; Fowler et al., 1999).

Rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a custom stereotaxic head

holder in a prone position on the bed of the scanner. Animals were then injected via the tail vein with [11 C]raclopride ($334 \pm 76 \mu\text{Ci}$; specific activity, 1.4–7.0 mCi/nmol). Injected volumes were approximately 400 μl . Total acquisition time was 60 min (24 frames: 6 frames, 10 s; 3 frames, 20 s; 8 frames, 60 s; 4 frames, 300 s; 3 frames, 600 s), and data were acquired in fully 3-dimensional mode with maximum axial acceptance angle ($\pm 28^\circ$). Images were constructed using Fourier rebinning (Matej et al., 1998) followed by 2-dimensional filtered back-projection with a ramp filter cutoff at Nyquist frequency.

2.3.1.2. MRI imaging. For accurate qualitative and quantitative image analysis using μ MRI- μ PET image coregistration, a MRI template image from an aged-matched Sprague–Dawley rat was acquired on a 9.4T Bruker Biospin Avance scanner (Bruker Biospin Corporation, Massachusetts, USA) using the RARE pulse sequence with an acceleration factor of 8 and TE/TR=40/2600 ms. The matrix size was 256×256 points at a Field of View (FOV) of $3.84 \times 3.84 \text{ cm}^2$, which gave an in plane resolution of

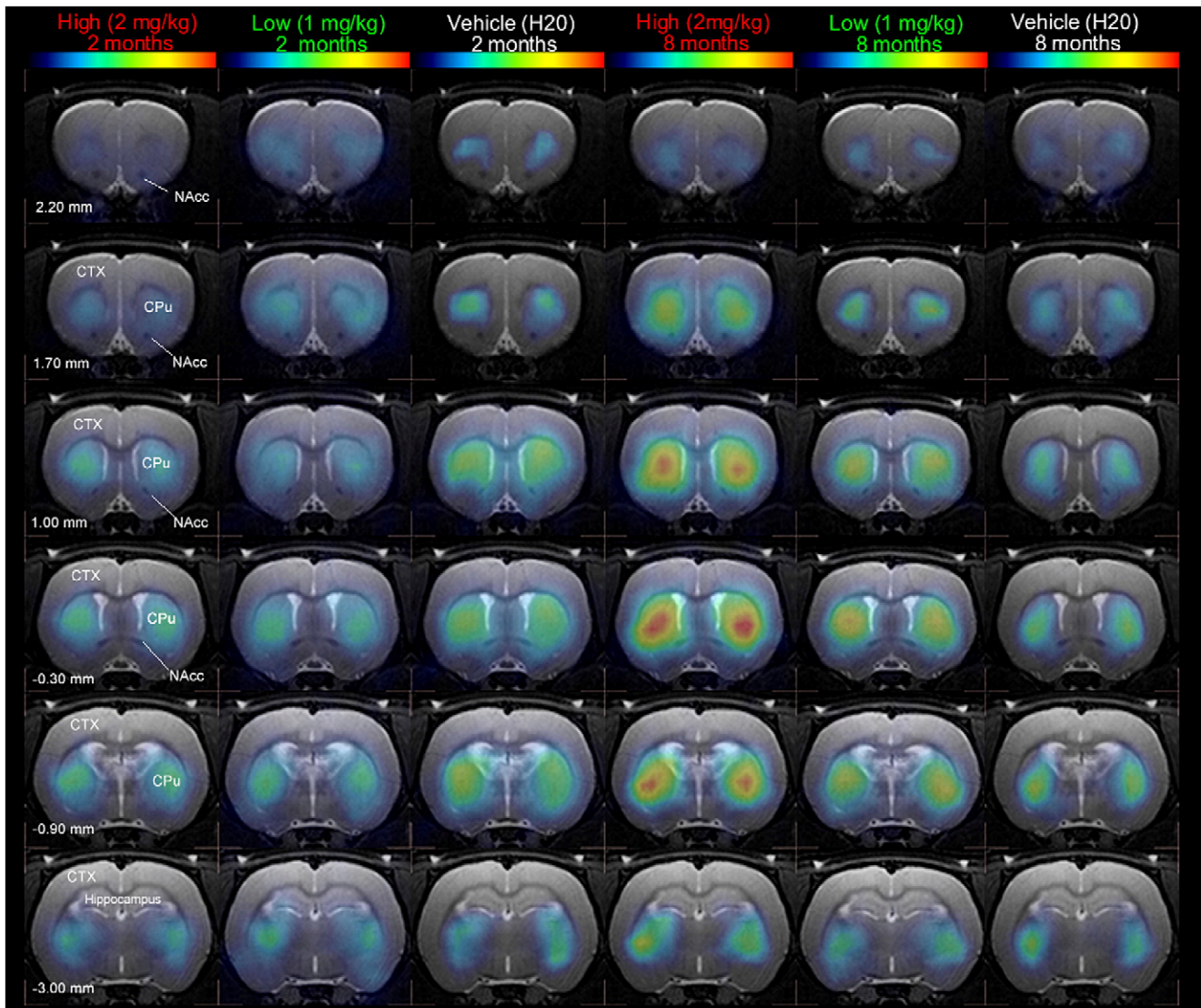


Fig. 1. Representative [11 C]raclopride μ PET images across groups following chronic oral treatment with MP or vehicle.

150 μm . The slice thickness was 0.9 mm with a 0.1 mm gap between slices; and the total acquisition time was 11 min.

2.3.1.3. μPET Analysis. Using the Pixel-Wise Modeling Software Suite (PMOD Technologies, Switzerland), μPET images were co-registered with the previously generated μMRI template. Initially the two images were co-registered using the method of Mutual Information (MI) (Woods et al., 1992), (Woods et al., 1993); through a MI algorithm implemented within the PMOD environment, followed by a manual adjustment of the μPET image in all three planes (sagittal, coronal, transversal) so that the Harderian Glands (HG) and caudate putamen from both imaging modalities matched. Using the Region of Interest (ROI) method; ROIs were selected for the left and right striatum (ST) and the cerebellum (CB) using the HG as a reference point. Specifically the ST and CB for each animal were identified as 8 and 12 slices respectively, caudal to the HG (slice thickness, 1.2 mm). It has been shown previously that the HG (located just rostral of the brain), because of their uptake of radioactivity, are useful markers in rodent PET studies (Thanos et al., 2002; 2004; Hume et al., 1996; Fukuyama et al., 1998; Kuge et al., 1997).

Qualitative assessment of μPET images was performed, and representative μPET images of each rat were acquired. The image analysis was performed using the Fusion, PxMOD and Kinetic programs included in the PMOD software suite (PMOD Technologies, Zurich, Switzerland). Quantitative analysis of the μPET images consisted of the MRTM0 (Multilinear Reference Tissue Model) Ichise Binding Potential (BP) which has been shown to be a reliable measure of D2R receptor occupancy (Ichise et al., 2003).

2.3.2. Experiment 2. Cocaine self-administration

Surgery was performed prior to operant training on animals at 9 months of age. Rats were anesthetized with 100/10 mg/kg Ketamine/Xylazine (Fort Dodge, TX/Lloyd Laboratories, IA). A 4-centimeter lateral incision was made along the chest and the right jugular vein was dissected out and isolated from the surrounding tissue. A 20-gauge heparin tipped, silicon catheter (Instech Solomon, PA) was implanted into the jugular vein and anchored to the surrounding fascia with a 7–0 silk suture (Biosurgery, MA). The venous line was flushed with normal saline to test patency and to compensate for volumetric blood loss. The distal catheter tubing was subcutaneously routed to the dorsal region of the animal, where it was attached to a back-mounted 22-G cannula (Plastics One, VA). The line was flushed daily with saline and filled with heparin (80 IU/ml) /cefazolin (200 mg/ml) locking solution (Sigma, USA/G.C. Hanford, NY) to prevent clotting and thrombus formation. Animals were given a one-week recovery period before they are started on the operant conditioning task.

After recovery, animals were put on a fixed-ratio food-training schedule using operant boxes (Coulbourn Instruments, PA) until animals reached a stable FR4 lever pressing for food baseline (achieved a criteria of <20% variation in the mean number of food pellets for 3 consecutive days) before being started on cocaine self-administration.

Cocaine self-administration (1 mg/kg/0.1 ml infusion) sessions were carried out under a FR1 schedule, with a 30 second time out period between consecutive reinforcements (increased from 5 s time out for food). Session duration was always 2 h, and rats were run daily for 15 days. Self-administration data was acquired using Graphic State software (Coulbourn Instruments, PA). Cumulative lever-press and drug infusion data were recorded for each session.

3. Results

3.1. Experiment 1

3.1.1. Weight

Mean weight across each treatment group was assessed after 8 months of treatment. A one-way ANOVA revealed no significant differences in mean weight between groups ($F=0.383$; $df=2.126$; $p=\text{ns}$; mean weights by group corresponded to: 395 + 18 g (2 mg/kg); 400 + 16 g (1 mg/kg); and 413 + 21 g (Vehicle).

3.1.2. D2R μPET Imaging

Fig. 1 shows the [^{11}C]raclopride images for representative groups of rats at 2 and at 8 months of treatment with either MP or vehicle. A two-way ANOVA and subsequent Holm–Sidak multiple comparison procedures were used to examine D2R availability at 2 and 8 months of treatment. In order to maximize statistical power, statistical observations used in the ANOVA consisted of the individual ROI's of each brain. A statistically significant main effect was observed with respect to treatment ($F=5.535$; $df=2.239$; $p=0.004$), time ($F=9.268$; $df=1.239$; $p=0.003$), and in the interaction between these two factors ($F=38.995$; $df=2.239$; $p<0.001$). There were no significant differences in D2R binding between the 1 and the 2 mg/kg dose treated rats after 2 months of treatment. However, pairwise comparisons during this time revealed significantly lower D2R binding between the 1 mg/kg dose ($t=4.237$; $p<0.001$) and the

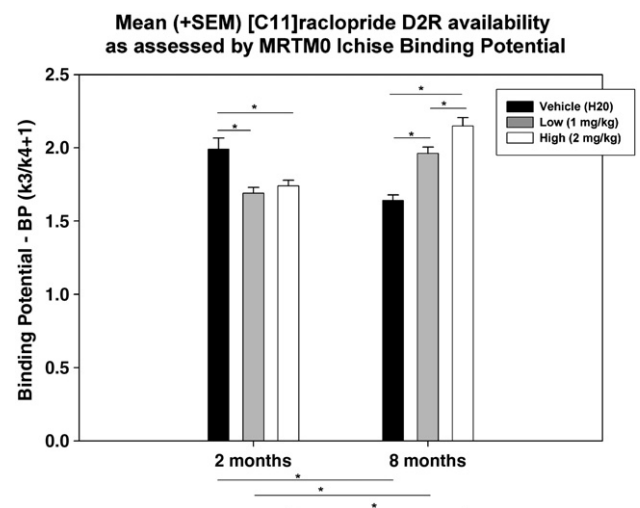


Fig. 2. Mean (+SEM) [^{11}C] raclopride D2R binding levels using the MRTM0 Ichise Binding Potential (BP) across treatment and age groups. * denotes significant differences as outlined in the figure with a p value equal to or less than 0.001 using Holm–Sidak pairwise comparisons.

2 mg/kg dose ($t=3.516$; $p=0.001$) treated rats as compared to the age-matched vehicle group (Fig. 2). D2R availability was shown to have increased for both the 2 mg/kg ($t=6.847$; $p<0.001$) and the 1 mg/kg dose ($t=4.567$; $p<0.001$) treated groups in a dose-dependent manner from 2 to 8 months of treatment while it decreased in the vehicle treated rats ($t=5.200$; $p<0.001$) (Fig. 2). Furthermore, after 8 months of treatment the group treated with 2 mg/kg showed greater D2R binding availability compared to the group treated with 1 mg/kg ($t=3.393$; $p=0.001$) and the vehicle treated group ($t=9.225$; $p<0.001$). Finally, after 8 months of treatment, the group treated with

1 mg/kg showed greater D2R binding compared to the vehicle treated group ($t=5.832$; $p<0.001$).

3.2. Experiment 2

There were no differences between groups in self-administration behavior during operant training. During cocaine self-administration sessions, MP treated rats displayed significantly lower mean active lever responses compared to vehicle treated rats. A one-way ANOVA revealed significant differences between groups ($F=162.754$; $df=2.33$; $p<0.001$). Subsequent pairwise comparisons (Holm–Sidak) between the 3 groups revealed that there was a significant difference in the mean number of active lever responses between the 2 mg/kg and 1 mg/kg MP group ($t=16.608$; $p<0.01$) and between the 2 mg/kg and vehicle treated rats ($t=14.413$; $p<0.05$) (Fig. 3). There were no significant differences in active lever responses between 1 mg/kg and vehicle treated animals. The rats treated with 2 mg/kg pressed the lever significantly less than the animals given 1 mg/kg MP or the vehicle treated animals.

Cocaine intake was examined in terms of the number of cocaine infusions. A one-way ANOVA between groups for mean total number of infusions revealed a significant difference ($F=30.966$; $df=2.33$; $p<0.001$; Fig. 3). Subsequent pairwise comparisons (Holm–Sidak) between the 3 groups revealed that 2 mg/kg MP treated rats had significantly lower number of infusions than both the 1 mg/kg MP ($t=6.925$; $p<0.05$) and the vehicle groups ($t=6.714$; $p<0.05$). There were no differences in the number of cocaine infusions between the rats treated with 1 mg/kg and the vehicle treated rats.

4. Discussion

This is the first study to evaluate the effects of 8 months of treatment with oral MP in the rat. Previous studies had limited the duration of MP to 2 weeks and concentrated on assessing the effects at one point in adulthood. Also different from other studies this is the first to evaluate the effects of chronic MP treatment on D2R availability. We show that daily oral treatment with MP initiated during the period corresponding to adolescence in the rat resulted in: (1) a decrease of striatal D2R availability at 2 months of treatment and an increase at 8 months of MP (2 mg/kg and 1 mg/kg) treatment. (2) a decrease in the self-administration of cocaine following 8 months of MP (2 mg/kg) treatment.

4.1. Effects of chronic MP after 2 months of treatment

Our imaging data after 2 months of MP treatment showed a significantly lower D2R availability in both MP treated groups of rats relative to age-matched vehicle-treated rats. The reduction in D2R availability could reflect downregulation of D2R but since [^{11}C]raclopride competes with DA in binding to D2R it could also reflect an increase in synaptic DA. Future studies are required to assess if there are changes in DA release after 2 months of MP treatment given at this developmental stage. Also, it is important to examine possible neuroadaptations in

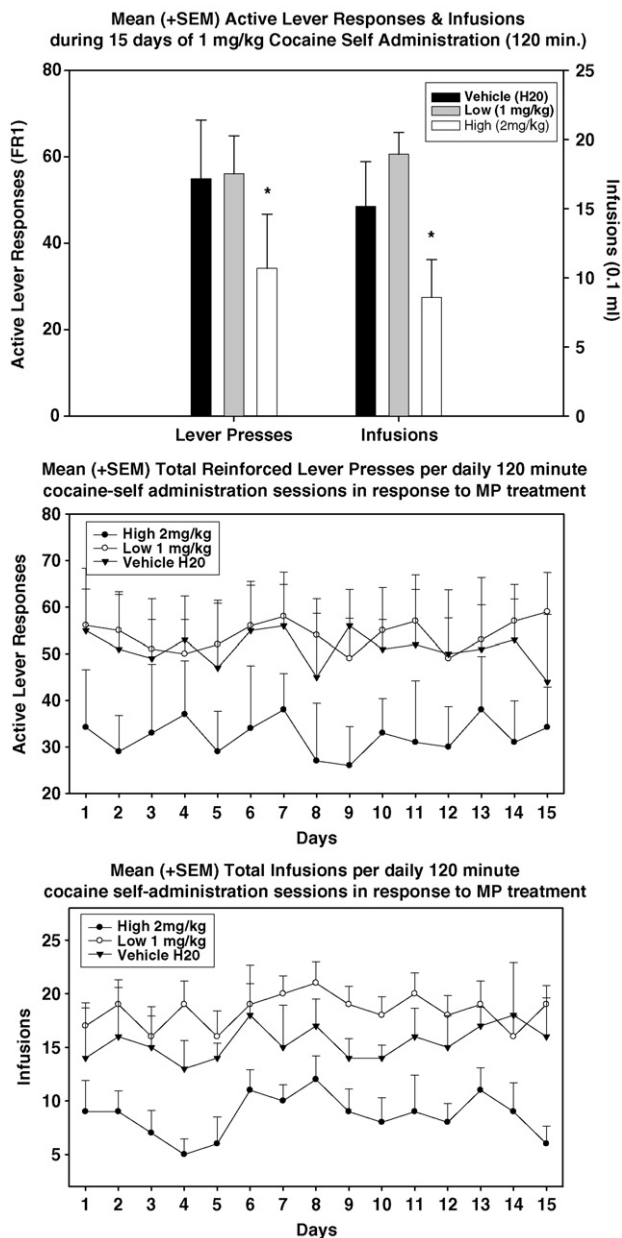


Fig. 3. A. Mean number (+SEM) of active lever responses and subsequent infusions across treatment and age groups. * denotes significant differences between the high (2 mg/kg) MP dose and the other two treatments with a p value less than 0.05 using Holm–Sidak pairwise comparisons. B. Mean Reinforced Lever Presses across session. C. Mean Cocaine Infusions across session.

other neurotransmitter systems, such as the noradrenergic system (Shaywitz et al., 1982; Wargin et al., 1983; Swanson et al., 1999; Vitiello, 2001).

4.2. Effects of Chronic MP after 8 months of treatment

In contrast to the decrease in D2R availability observed after two months of MP treatment, animals tested after 8 months of treatment showed increased D2R availability when compared with animals treated with vehicle. The differences in the results obtained at 2 and 8 months are likely to reflect the difference in the duration of treatment. Furthermore, it is possible that these results also reflect differences on the effects of chronic MP dose and as a function of the developmental stage of the animal (young adolescence versus young adulthood), but this remains to be substantiated since we did not use a similar treatment regiment in adult animals. Finally, these differences may also be related to changes in synaptic DA concentrations, which can compete with [¹¹C]raclopride for D2R binding sites. Previous studies have also discussed the potential effects of DA on D2R internalization, making the receptor unavailable for binding with [¹¹C]raclopride (Laruelle, 2000; Sun et al., 2003). Eight months of treatment with MP was also shown to significantly reduce cocaine-self administration for the 2 mg/kg dose though there was no effect for the 1 mg/kg MP dose. Unfortunately, we can not compare cocaine self-administration following 8 month MP treatment with that obtained after 2 months of treatment since animals were not tested (for cocaine self-administration) at this earlier time point to avoid confounds from potential long term effects secondary to cocaine exposure during this early developmental stage.

4.3. Significance of MP induced changes in D2R and cocaine self administration

In clinical studies we and others have shown decreases in D2R in substance abusers (cocaine, methamphetamine, alcohol, and heroin; reviewed (Volkow et al., 2004). Also we had shown that in non-drug abusing subjects D2R availability modulates the rewarding responses to intravenous MP. Specifically, subjects with low D2R availability tended to report MP as pleasant, whereas those with high D2R availability tended to report it as unpleasant. These findings have led us to hypothesize that low D2R availability in striatum increases the vulnerability whereas high D2R protect against substance abuse (Volkow et al., 2004). Indeed in rodents trained to self-administer alcohol, D2R overexpression in nucleus accumbens resulted in marked reductions in alcohol intake both in rats that were genetically predisposed to self administering alcohol (Thanos et al., 2004), as well as in those that were not (Thanos et al., 2001). An inverse relationship between D2R availability and vulnerability to the reinforcing effects of cocaine (Nader and Czoty, 2005) and amphetamine (Ginovart et al., 1999) has also been reported in nonhuman primates. Morgan and colleagues have shown that social environmental interventions that lead to increases in D2R availability led to decreases in cocaine self-administration (Morgan et al., 2002) whereas interventions that resulted in low D2R availability led to a

facilitation in cocaine administration (Morgan et al., 2002). Moreover a recent study documents an inverse relationship between D2R availability and propensity to self-administer cocaine (Nader et al., 2006). Thus, these studies provide indirect evidence that the high D2R availability in animals treated chronically with MP could underlie their attenuated administration of cocaine. Our data showing increases in D2R availability at 8 months of treatment would therefore suggest that chronic MP treatment (started in adolescence) attenuated cocaine self-administration during adulthood. Indeed the lower rates of cocaine self-administration in the animals treated for 8 months with MP (2 mg/kg ip) support this. In contrast, the lower D2R availability at 2 months of MP treatment would suggest that shorter lengths of treatment or the age at which the treatment effects are evaluated (early versus late adulthood) could result in different effects. In this case a lowering of D2R availability could make the animals more vulnerable to drug self-administration during early adulthood (after 2 months of treatment). Unfortunately, we did not evaluate cocaine self-administration at this time point which would have allowed us to corroborate if low D2R availability was associated with increased cocaine consumption. Prior studies in rodents had reported that 2 weeks of MP treatment led to a reduction in cocaine and methamphetamine preference (Carlezon et al., 2003; Kuczenski and Segal, 2002; Andersen et al., 2002) but another study reported that it increased cocaine self-administration (Brandon et al., 2001). These apparent discrepancies are likely to reflect a) differences in the stage of development when MP was given (pre-adolescence versus adolescent rats); b) the different tests used to assess cocaine's rewarding effects (conditioned place preference versus self-administration) and c) the route of administration of MP (ip versus oral). On the other hand, clinical studies suggest that there is no increased risk for stimulant abuse associated with ADHD stimulant pharmacotherapy (Biederman and Spencer, 1999; Loney et al., 2002). The findings from this study pertain to the question of whether clinical treatment with stimulants in adolescence increases the risk for drug abuse in young adulthood. While our findings would support a reduction in the risk for cocaine self-administration we have to be cautious since drug intake in the real life situation is much more complex than in a laboratory setting. Specifically in real life drug intake reflects a selection among various competing reinforcers and thus changes in the sensitivity of reward centers in the brain not only to cocaine but also to other rewards could still result in an increase risk for drug abuse. This is relevant since prior studies have shown that chronic MP not only decreases the sensitivity to the rewarding properties of cocaine but also to that of natural reinforcers (Bolanos et al., 2003). It has been proposed that the therapeutic effects of psychostimulants in ADHD reflect in part their ability to enhance the saliency of reinforcers while they exert their pharmacological effects (Solanto 1998; Wilkison et al., 1995).

4.4. Study limitations

The present study examined D2R availability in rats longitudinally after 2 and 8 months of MP treatment however we

only examined cocaine self-administration in these same rats after 8 months of MP treatment. Thus one limitation to our study was that we did not evaluate cocaine self-administration after 2 months of MP treatment. Future experiments will examine the relationship between the low D2R availability observed after 2 months of treatment with cocaine self-administration. More studies evaluating the effects of different lengths of MP treatment on drug self-administration are required to assess optimal duration of treatment regimes to minimize adverse effects on the propensity for drug self administration.

In this study we only tested one dose of cocaine and thus we can not rule out the possibility that the decrease in cocaine-self administration in the MP treated rats (2 mg/kg) was due to an enhanced sensitivity to the rewarding effects of cocaine (thus animals required less of the drug) rather than a decrease in the reinforcing effects of the drug. This is because cocaine self-administration is characterized by an inverted U-shaped dose response curve (Sizemore et al., 1997) where the interpretation of decreased rewarding effects of cocaine would depend on where one is on the curve. However, regardless of the mechanism(s) responsible for the decrease in cocaine self-administration (i.e. decrease rewarding properties of cocaine or an increased sensitivity to its reinforcing effects) the net result was a significant decline in cocaine consumption. Though unlikely, since there is no evidence that chronic treatment with MP in ADHD induces withdrawal upon discontinuation, we cannot rule out the possibility that withdrawal may have affected the responses to cocaine.

When assessing D2R availability using μ PET, we used ketamine as an anesthetic. There is some discussion as to whether or not ketamine binds to the D2R in-vivo. It has previously been shown that surgical concentrations of isoflurane, halothane, ethanol and ketamine inhibit high affinity states of the D2R as well as other G-protein linked receptors in-vitro (Kapur and Seeman, 2002; Seeman and Kapur, 2003). We do not feel that this raises a major concern for our data since all animals were administered ketamine during the scanning period and therefore any effects on the D2R would apply to all groups. However, this does raise the question of whether or not there were any interactions between ketamine, [11 C]raclopride, MP exposure and age in our experiment. Future studies using the above anesthetics should take this into account.

It is important to point out that the findings from this study cannot be directly extrapolated to the treatment regimes used for ADHD since in general these are of shorter relative duration. However, what this paper addresses is the effects of long-term treatment with MP in DA function of the adult brain. It is also important to point out that the current and other preclinical studies are done in healthy animals and not in rodent models of ADHD.

4.5. Summary

This study provides evidence that although chronic MP treatment during the adolescent–adulthood transition may lead to changes in striatum D2R levels, that have been associated with a greater vulnerability to drug abuse (D2R decreases), over

the long-term, MP treatment may produce an increase in D2R availability which in this study was associated with an attenuation of cocaine self-administration. This study also documented a reversal of the age associated reduction in D2R with chronic MP that deserves further investigation to evaluate its merits as a potential intervention to help prevent the reductions in D2R that occurs with age, which have been shown to contribute to the decline in locomotor and cognitive function in the elderly (Volkow et al., 1998).

Acknowledgments

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