Adolescent Exposure to Methylphenidate Alters the Activity of Rat Midbrain Dopamine Neurons

Cindy L. Brandon, Michela Marinelli, and Francis J. White

Background: *Methylphenidate is commonly used to treat children and adolescents with attention-deficit/hyperactivity disorder. A health concern is its long-term effects with respect to later stimulant exposure. We reported that repeated exposure to a low dose of methylphenidate during adolescence increases self-administration of a low, typically nonreinforcing dose of cocaine in adult rats. We also showed that enhanced vulnerability to cocaine is associated with elevated impulse and bursting activity of midbrain dopamine neurons in drug-naïve adult rats and might constitute a substrate critically associated with abuse liability. Thus we sought to determine whether repeated exposure to low-dose methylphenidate in adolescence alters dopamine neuronal excitability in adulthood.*

Methods: *After 3-day and 2-week withdrawal from repeated low-dose adolescent exposure to methylphenidate, we used extracellular single-unit recording in chloral hydrate–anesthetized rats to determine basal firing and bursting activity of midbrain dopamine neurons and dopamine autoreceptor sensitivity to the D2-class direct receptor agonist quinpirole.*

Results: *Dopamine neuronal impulse activity was increased after 3 days and decreased after 2 weeks' withdrawal from methylphenidate given in adolescence. No difference between groups was evident with respect to autoreceptor sensitivity to quinpirole.*

Conclusions: *Adolescent exposure to methylphenidate induces neuronal changes associated with increased addiction liability in rats.* Biol Psychiatry 2003;54: 1338–1344 © *2003 Society of Biological Psychiatry*

Key Words: Methylphenidate, Ritalin, cocaine, dopamine, addiction, electrophysiology

Introduction

In the year 2000, approximately 20 million monthly prescriptions for analeptic medications were written for the treatment of attention-deficit/hyperactivity disorder (ADHD). The most common analeptic, methylphenidate, the generic form of the brand-name drug Ritalin (National Institute on Drug Abuse, Rockville, Maryland), topped both prescriptions and sales (Jensen et al 2001; Shaywitz et al 2001). Recommendations for the continued and expanded use of stimulants followed the Multimodal Treatment Study report, which documented that stimulant pharmacotherapy was superior to behavioral therapy for the vast number of children diagnosed with ADHD (Jensen et al 2001). Increased funding for clinical trials for the psychopharmacologic use of stimulants in preschoolers (Vitiello 2001) and a report identifying recipients of stimulants who do not meet the diagnostic criteria for ADHD (Marshall 2000) further suggest that stimulant use might continue to increase in coming years.

Although the therapeutic utility of stimulants in treating ADHD is undeniable, a major concern is that long-term stimulant administration in children and adolescents might alter the brain in manners that affect subsequent responses to stimulants or other drugs with potential for abuse (Vitiello and Burke 1999). Reports show that adolescence is a critical developmental period, during which drug exposure might lead to increased addiction (Laviola et al 1999). These factors indicate that methylphenidate exposure for protracted periods in childhood and adolescence might constitute a potential public health concern. Clinical reports regarding the effects of adolescent stimulant exposure on subsequent drug use remain controversial (for example, see Lambert 2002; Wilens et al 2003).

Psychomotor stimulant sensitization in animal models of addiction correlates with neuroadaptations in the brain associated with increased abuse liability (for review, see White and Kalivas 1998). Although methylphenidate produces context-dependent locomotor sensitization in adult animals after repeated doses as low as 1.0 mg/kg (Kuczenski and Segal 2001), we recently reported that repeated exposure to a low dose of methylphenidate (2.0 mg/kg, IP) during adolescence does not produce heightened motor activation to cocaine challenge over a wide range of doses

From the Departments of Anatomy and Cell Biology (CLB) and Cellular and Molecular Pharmacology (MM, FJW), Finch University of Health Sciences/The Chicago Medical School, North Chicago, Illinois.

Address reprint requests to Dr. Francis J. White, Finch University of Health Sciences, Chicago Medical School, Department of Cellular and Molecular Pharmacology, 3333 Green Bay Road, North Chicago IL 60048.

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in young adult rats and therefore does not cross-sensitize to the psychomotor effects of cocaine (Brandon et al 2001). This same treatment protocol, however, resulted in enhanced self-administration of a low, typically nonreinforcing dose of cocaine (75 μg/kg/infusion) in the methylphenidate-withdrawn (2 weeks) and now young adult rats. Thus, there is dissociation between the psychomotor stimulant effects of cocaine and its ability to support self-administration after adolescent exposure to methylphenidate. We concluded that methylphenidate-induced increased vulnerability to cocaine self-administration might represent generalized risk to individuals currently exposed to this and other stimulants. Indeed, early exposure to methylphenidate has been associated with increased lifetime use of nicotine (Lambert 2002; Lambert and Hartsough 1998) and cocaine in humans (Barkley et al 2003; Schenk and Davidson 1998; but see Wilens et al 2003).

At the cellular level, withdrawal from repeated noncontingent administration of psychomotor stimulants produces a transient increase in the basal activity of dopamine neurons within the ventral tegmental area as a result of decreased sensitivity of impulse-regulating D2 autoreceptors (see White 1996 for review); similar results have been reported for cocaine self-administration (Marinelli et al 2003). These effects are relatively transient but are necessary for the development of behavioral sensitization to stimulants. In addition, we have recently reported that enhanced individual vulnerability to cocaine self-administration, in drug-naïve adult rats, is associated with elevated impulse activity of midbrain dopamine neurons (Marinelli and White 2000). Thus, we concluded that increased basal activity might constitute a substrate critically associated with abuse liability. In this study, we sought to determine whether repeated exposure to a low dose of methylphenidate in adolescence would likewise alter dopamine neuronal activity in young adulthood.

Methods and Materials

Subjects and Treatments

Four-week-old male Sprague-Dawley rats (Harlan, Indianapolis, Indiana) were housed individually and allowed 1 week for acclimation to the vivarium (12:12 light/dark; lights on 7:00 AM) under constant humidity (40%–80%), temperature (21–23°C), and ad libitum food and water. Rats were randomly assigned to experimental groups. They were injected IP in their home cages with either a saline vehicle (.9% sodium chloride [NaCl]) or methylphenidate (National Institute on Drug Abuse) (2.0 mg/kg, dissolved in saline) for 7 days (3–5 hours before lights off, one time/day), and allowed 1–3 days or 14–21 days (during which they were handled and weighed twice) before electrophysiologic recordings. This withdrawal period parallels the same period during which methylphenidate-treated adolescent rats were tested for cocaine self-administration (Brandon et al 2001). The experimenter was always blind to treatment conditions.

Extracellular Single-Unit Recording

After early (1–3 days) or late (14–21 days) withdrawal periods, rats were anesthetized with chloral hydrate (400 mg/kg, IP) and mounted in a stereotaxic apparatus (Activational Systems, Warren, Michigan) with the incisor bar set 3 mm below the interaural line. A lateral tail vein was catheterized with a 25-gauge hypodermic needle to administer additional anesthetic or drugs (as required). Body temperature was monitored by a rectal thermometer and maintained at 36.5–37.0°C with a thermostatically controlled heating pad (Fintronics, Orange, Connecticut). A burr hole was drilled in the skull, and the dura mater was retracted from the area overlying the ventral tegmental area (VTA). A glass electrode was pulled from 2.0- or 1.5-mm outer-diameter glass tubing with a vertical electrode puller (Narishige PE-2, Tokyo, Japan), broken back under a microscope to a tip diameter of 1–2 μm, and filled with a 2 mol/L NaCl solution saturated with 1% fast green dye (Fisher Scientific, Houston, Texas). The electrode was lowered to 2 mm above the VTA and then slowly advanced with a hydraulic microdrive (David Knopf Instruments, Tujunga, California) to the dopamine cell body region. The coordinates for the VTA were 3.0–3.8 mm anterior to lambda, .3–.7 mm lateral from the midline, and 7.5–8.5 mm ventral to the cortical surface (Paxinos and Watson 1986). In vitro impedance of the electrodes was 1.5–2.1 MΩ, measured at 135 Hz (Winston Electronics BL1000-B, San Francisco, California). During extracellular recording, electrical signals were fed into a high-impedance amplifier (Fintronics), filtered at 400 and 500 Hz, displayed on an oscilloscope (Tektronix, 5110, Chicago, Illinois) and monitored by a window discriminator and an audio amplifier (Grass AM8, Quincy, Massachusetts). The analog output of the window discriminator was connected to a polygraph recorder (Gould 220, Chicago, Illinois) that plotted the rate histograms and to a printer (DPP-Q7A1; Datel, Mansfield, Massachusetts) that printed firing rates. Digital outputs were led through an interface (Digidata 1200 series; Axon Instruments, Foster City, California) to a personal computer running AxoScope software (Axon Instruments) that determined firing activity online and stored all data for future analysis. Stored data were then analyzed with a custom-made program that determined firing and bursting activity.

Dopamine cells were identified by anatomic location in the VTA and according to standard physiologic criteria (White 1996). These neurons had 1) a characteristic triphasic (+/−/+) waveform with a long action potential of 2.5–3.5 msec; 2) low spontaneous firing rates of .5–10 Hz; and 3) either a slow irregular firing pattern or a slow bursting pattern with decreasing spike amplitude and increasing interspike interval within the burst. Ventral tegmental area dopamine neuronal activity was measured from one to four cells per rat. Cells were recorded between 3 and 5 min to establish a mean baseline firing rate. Only cells with stable activity over at least 3 min (<3% variation unless firing was less than 2 Hz, then <5% variation) were included. Bursting activity was plotted as percentage of spikes emitted in bursts. Burst events were initiated by a pair of spikes having an interspike

interval ≤ 80 msec and terminated by interspike intervals ≥ 160 msec (Grace and Bunney 1984a, 1984b).

Autoreceptor Sensitivity Test

The response of dopamine neurons to intravenous administration of the dopamine D2-class receptor agonist quinpirole was used as a measure of autoreceptor sensitivity (White and Wang 1984a). After 3 min of stable firing, quinpirole was administered through the catheterized tail vein using a cumulative dosing regimen in which each dose doubled the previous one at 60–90 sec intervals. Since the experimenter was blind to the treatment groups, we generally chose cells with similar firing rates. The dopamine D2-class receptor antagonist eticlopride (.1 mg/kg, IV) was administered to reverse agonist-induced inhibition. Only one cell was recorded per rat.

Dose Response for Methylphenidate

The same procedure for obtaining a stable cell and a cumulative dose regimen (starting with 100 μg/kg methylphenidate) was used to determine the effects of methylphenidate (IV) on the activity of VTA dopamine neurons in drug-naïve rats.

Histology

At the end of recording, passing a 28-μA cathodal current through the electrode for 20–45 min marked the position of the electrode tip. This deposited a discrete dye spot. Rats were then deeply anesthetized with additional chloral hydrate and perfused transcardially with .9% NaCl followed by 10% formalin. Brains were stored in 10% formalin until serial coronal sections were cut on a freezing microtome (American Optical, Buffalo, New York). Sections were then mounted and stained with cresyl violet, and electrode placement was confirmed with a light microscope.

Statistical Analysis

T tests were performed between saline control groups at both withdrawal times for each dependent variable. The following dependent variables were considered: firing rate (Hz), percent bursting in spikes, interspike interval (msec), burst frequency, and spikes per burst. Saline groups from both withdrawal times were combined because there was no significant difference between them for each of these measurements [$t(79) = -.06$, .34, .88, .46, .71, ns, respectively]. Then *t* tests were performed between each methylphenidate withdrawal group and the pooled controls. The effects of quinpirole were also analyzed with repeated-measures analysis of variance (ANOVA), with the dose of quinpirole used as the within factor (11 levels, doses 0–512). Cells from each methylphenidate withdrawal group were compared with saline controls from the same withdrawal period.

Results

VTA Dopamine Neuronal Response to Methylphenidate in Adolescent Rats

Figure 1 shows the ability of methylphenidate to inhibit dopamine neurons in the VTA of adolescent, chloral hydrate–anesthetized rats. Using single-unit extracellular recording, we found that the VTA dopamine neuronal response to IV methylphenidate was consistent with other dopamine uptake inhibitors, partially inhibiting basal firing rate by 66% (Einhorn et al 1988) at a cumulative IV dose as high as 6.4 mg/kg. Intravenous doses much higher than those corresponding to doses used clinically and orally were unable to completely inhibit cell firing and inhibited neurons by less than 20% when in the therapeutic range (.4–2 mg/kg oral route).

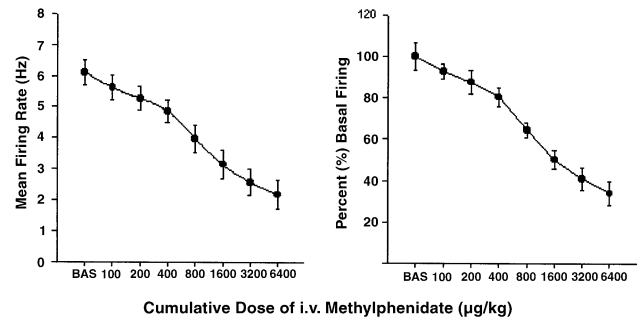

Figure 1. Methylphenidate suppresses the activity of ventral tegmental area dopamine neurons in 5–6-week-old rats. Intravenous administration of methylphenidate caused a significant dose-dependent, partial inhibition (66%) of firing of identified mesoaccumbens dopamine neurons. Each point represents the mean ± SEM ($n = 8$ cells and animals).

VTA Dopamine Neuronal Activity after Methylphenidate Withdrawal

Figure 2 shows that dopamine neurons in the short-withdrawal group showed a significantly higher mean firing rate compared with the pooled saline control group. Burst analysis showed that there was a trend for an increased percentage of spikes emitted in bursts in the short-withdrawal group (Figure 3) and a significantly increased frequency of burst events. In addition, neurons from the methylphenidate short-term withdrawal group showed significantly more average spikes per burst (Figure 3) compared with control animals. Ventral tegmental area dopamine neuronal activity in vivo was significantly attenuated after longer withdrawal (14–21 days) compared with all saline-preexposed animals. This was measured by both lower mean firing rate and greater average interspike interval (Figure 2). In addition, there was a trend ($p < .06$) in the long-term withdrawal group for a decreased average number of spikes emitted per burst (Figure 3) compared with control animals.

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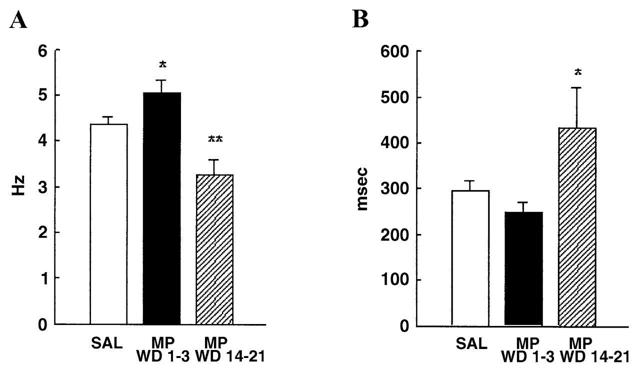

Figure 2. Methylphenidate treatment in adolescence differentially alters the firing rate of ventral tegmental area (VTA) dopamine neurons at two withdrawal times. After 3 days' withdrawal from methylphenidate (2 mg/kg, IP for 7 days) the firing rate of VTA dopamine neurons **(A)** was significantly higher in methylphenidate-treated rats compared with saline-pretreated control rats [$t(131) = -2.16$, $*p < .05$ (for saline: mean = 4.35 ± .19 Hz, $n = 81$ cells, 38 rats; for methylphenidate withdrawal 1–3 days: mean = 5.05 ± .27 Hz, $n = 52$ cells, 25 rats)]. After a longer withdrawal from methylphenidate (14–21 days), the now young adult rats showed significantly decreased firing [mean = 3.26 ± .34 Hz, $n = 22$ cells, 12 rats; $t(101) = 2.69$, $**p < .01$]. In addition, the interspike interval **(B)** was significantly increased in the long-term withdrawal group [$t(101) = 2.23$, $*p < .05$] compared with control animals (mean = 434.21 ± 85.87 msec and 295.10 ± 22.90 msec, respectively) (right panel). MP, methylphenidate; WD, withdrawal; SAL, saline.

VTA Dopamine Neuronal Autoreceptor Sensitivity after Methylphenidate Withdrawal

Autoreceptor-mediated inhibition of firing in VTA dopamine neurons was measured by use of cumulative dose-response curves for the dopamine D2-class direct receptor agonist quinpirole. Quinpirole induced a dose-dependent decrease in firing in both saline- and methylphenidate-exposed groups for both withdrawals, and there were no significant differences in sensitivity to quinpirole across groups (Figure 4). Similar results were observed when data were analyzed as a percentage of the initial baseline values (data not shown). In all cases, the dopamine D2-class antagonist eticlopride reversed the agonist-induced inhibition (data not shown).

Discussion

Our results demonstrate that methylphenidate, like other dopamine transporter blockers, can suppress the firing of VTA dopamine neurons, but only at relatively high doses and only with partial efficacy (Einhorn et al 1988; Hinerth et al 2000). More importantly, we showed that repeated low-dose treatment of methylphenidate in adolescent rats differentially affected the activity of VTA dopamine neuronal activity, depending on the length of withdrawal after the last treatment. We saw decreases in VTA dopamine neuronal activity after 2 weeks of withdrawal when rats had simultaneously reached young adulthood; however, after 3 days' withdrawal, we saw increased excitability of dopamine neurons in rats that were also still adolescent. Altered dopamine neuronal activity included changes in both firing rates and bursting activity, but these effects do not seem to be mediated by changes in sensitivity of impulse-regulating dopamine D2 autoreceptors.

Acute Response to Methylphenidate

Unlike direct dopamine D2 receptor agonists (White 1996), indirect dopamine receptor agonists that increase extracellular dopamine levels by blocking the dopamine transporter typically suppress the firing of VTA dopamine neurons only partially, unless very high doses are give (Einhorn et al 1988; Hinerth et al 2000). Methylphenidate shares this property with drugs such as the dopamine reuptake inhibitors cocaine and GBR 12909 (1-[2-[bis[4-fluorophenyl]methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride), but seems more similar to GBR 12909, given the reduced efficacy as compared with cocaine (Einhorn et al 1988; Hinerth et al 2000). From a clinical perspective, it is important to note that within a dose range (.4–2.0 mg/kg) that might be considered analogous to the proposed therapeutic dose range for methylphenidate (Gerasimov et al 2000), this drug would inhibit dopamine neuron firing by less than 20%. Given that nerve terminal dopamine release would only be marginally reduced as a result of this small suppression of firing, and that dopamine transporter inhibition at the terminals of dopamine neurons would be effectively suppressed, such doses of methylphenidate should greatly enhance dopamine transmission within the nucleus accumbens and other dopamine terminal fields, as has been shown with microdialysis (Gerasimov et al 2000; Volkow et al 1998, 2001). Moreover, these findings do not support the hypothesis that low doses of stimulants used in the treatment of ADHD act preferentially through autoreceptor-mediated processes to decrease extracellular dopamine and locomotor activity (Seeman and Madras 1998; Solanto 1998). Instead, our results support studies (Ruskin et al 2001) suggesting that drugs used in the treatment of ADHD probably act preferentially at nerve terminal dopamine transporters to elevate dopamine levels and alter behavior.

Effects of Repeated Exposure to Methylphenidate during Adolescence

When adolescent rats received repeated methylphenidate treatment on a regimen that enhances the subsequent

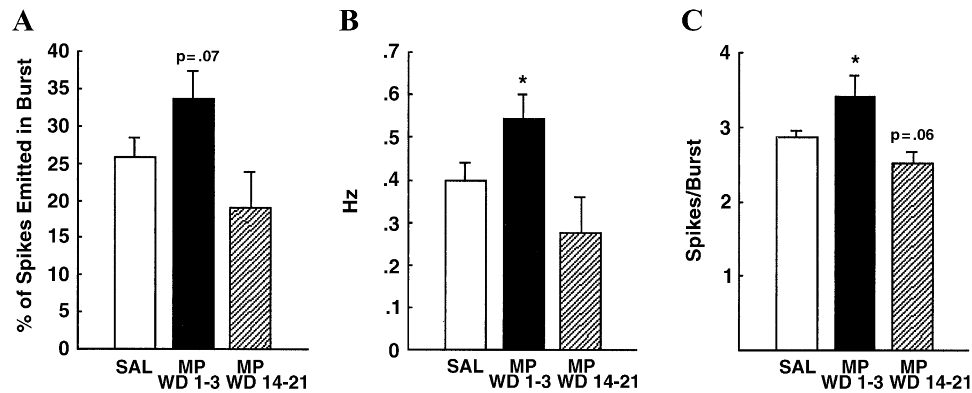

Figure 3. Bursting of ventral tegmental area (VTA) dopamine neurons is differentially altered after methylphenidate withdrawal. There was a trend for an increased percentage of spikes emitted in bursts **(A)** in dopamine neurons from the short-term withdrawal group compared with saline-pretreated control rats [$t(131) = -1.81$, $p = .07$ (for saline: mean = 26% ± 3%; for methylphenidate withdrawal 1–3 days: mean = 34% ± 4%)] and significantly more overall burst events **(B)** [$t(131) = 1.99$, *$p < .05$] in this group (mean = 5.42 ± .60 Hz) compared with control rats (mean = 3.98 ± .44 Hz). In addition, burst size **(C)** in VTA dopamine neurons was differentially modified after two different withdrawals from methylphenidate. There were significantly more spikes emitted in bursts in the short-withdrawal group compared with the control rats [$t(123) = -2.28$, *$p < .05$; for saline: mean = 2.87 ± .09; for methylphenidate withdrawal 1–3 days: mean = 3.42 ± .27 spikes/burst, respectively]. There was also a trend showing decreased spikes emitted in bursts in the long-term withdrawal group (mean = 2.51 ± .15 spikes/burst) compared with the control rats [$t(93) = 1.84$, $p = .06$.]. MP, methylphenidate; WD withdrawal; SAL, saline.

reinforcing but not psychomotor response to cocaine in young adulthood (Brandon et al 2001), we found that dopamine neuronal sensitivity to the dopamine D2-class agonist quinpirole was not altered during the first 3 days of withdrawal but that dopamine neuronal firing rate was higher. Why would this methylphenidate treatment not produce autoreceptor subsensitivity when similar treatments (White et al 1995; White and Wang 1984a) with other psychomotor stimulants do? It is possible that the lack of autoreceptor subsensitivity is due to treatment during adolescence, which has not been the case with other psychomotor stimulants. Dopamine D2 autoreceptors are in the process of development during this time (Laviola et al 1995; Ujike et al 1995), so that the effects of treatment during a critical period could alter their number or function. But it is more likely that it relates to the low doses of methylphenidate that do not enhance autoreceptor stimulation significantly and thereby fail to produce downregulation. In the case of the long-withdrawal group, the mean firing rate of sampled neurons in the quinpirole test did not reflect the lower average mean firing rate of this group. This might be due to methodology, whereby we chose neurons with higher mean firing rates not knowing that long-term withdrawal from methylphenidate would result in decreased neuronal activity. Had we sampled neurons with lower mean firing rates instead of expressly choosing neurons with similar firing rates (see Methods and Materials), they might have shown increased sensitivity.

When tested 14–21 days after the last methylphenidate injection, we observed a decrease in the activity of VTA dopamine neurons. This effect might be related to previous results obtained after 10–14 days of withdrawal from repeated intermittent cocaine administration in adult rats, which showed that there was attenuated cells/track measured in the VTA (Ackerman and White 1992). Microdialysis measurements of extracellular dopamine after long-term abstinence from repeated amphetamine or cocaine treatments also support a reduction in dopamine cell activity during sustained abstinence (Imperato et al 1992, 1996; Parsons et al 1991; Robertson et al 1991; Segal and Kuczenski 1992a, 1992b; Zhang et al 2001). Although acute stimulant-induced behavioral responses are normally dose-dependent and related to the magnitude of increases in dopamine, behavioral effects after chronic exposure to stimulants in these studies were often dissociated from the measurements of extracellular dopamine and persisted despite the waning dopamine response.

Our behavioral and electrophysiologic findings are consistent with recent reports (Ciccocioppo et al 2001; Grimm et al 2001; Neisewander et al 1996) demonstrating enhanced drug craving after longer withdrawals from drug exposure to cocaine, and therefore might indicate that low doses of stimulants in young rats induce changes in brain physiology that "incubate" over longer rather than shorter withdrawals, thereby progressively increasing risk to later exposure and enhancing vulnerability over time. Given that repeated exposure to low doses of methylphenidate has been shown to cause context-dependent behavioral sensitization (Kuczenski and Segal 2001), one has to conclude that even under conditions of prolonged absti-

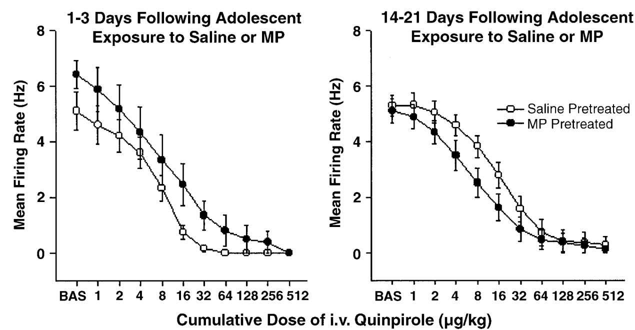

Figure 4. Midbrain dopamine neuronal sensitivity to the selective D2 receptor agonist quinpirole is not altered after methylphenidate treatment in adolescent rats. There was no significant difference in the dose response to IV quinpirole between treatment groups after 1–3 days abstinence from saline or methylphenidate [main effect: treatment $F(1,12) = 2.22$, $p = .1618$; treatment × dose of quinpirole interaction: $F(10,120) = .88$, $p = .5545$; $n = 7, 7$, for saline-pretreated and methylphenidate-pretreated groups, respectively). This was also true for the 14–21-day withdrawal groups [main effect: treatment $F(1,19) = 1.78$, $p = .1918$; treatment × dose of quinpirole interaction: $F(10,190) = 1.18$, $p = .3034$; $n =$ 11, 10 cells and animals for saline-pretreated and methylphenidate-pretreated groups respectively). MP, methylphenidate.

nence, previous drug exposure can cause "hyperincentive motivation" to be triggered by reward cues (Wyvell and Berridge 2001), thereby triggering hyperincentive "wanting" of even low doses of cocaine.

Consistent with studies in which animals were noncontingently treated with stimulants (White 1996; White and Kalivas 1998; White and Wang 1984b; White et al 1995), this study also showed that during early withdrawal from methylphenidate there were transient changes in midbrain dopamine neurons, including increased basal activity and bursting. Although these early changes in midbrain neurons do not endure into late withdrawal when enhanced drug responses are still present, they have been shown to be necessary (Kalivas and Stewart 1991; Vezina 1993; White and Kalivas 1998; Wolf et al 1994) for inducing cellular changes expressed downstream in other brain regions after longer withdrawal from stimulants. Changes in dopamine neurons after repeated treatment with methylphenidate in adolescence might reveal a particularly susceptible time during development when repeated exposure to even low doses of stimulants is not without risk.

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