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Behavioral reactivity to a noradrenergic challenge after chronic oral methylphenidate (Ritalin®) in rats

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Abstract

Methylphenidate (Ritalin®) is routinely used for the treatment of attention-deficit/hyperactivity disorder (ADHD). It is a psychomotor stimulant with pharmacodynamics similar to those established for cocaine and amphetamine with primary activation of the noradrenergic and dopaminergic systems. Long-term exposure to psychostimulants including methylphenidate (MPD) is believed to result in enduring functional changes along both these pathways and various behaviors mediated by these systems may be affected. In the present experiment, the effects of intermittent oral administration of methylphenidate (10 mg/kg) to rats over a 4-week period were subsequently (after a drug washout interval) assessed in three animal models sensitive to noradrenergic manipulation: the elevated plus-maze, predator odor avoidance, and social interaction tests. The behaviors of methylphenidate-experienced animals were compared with untreated controls. Thirty minutes prior to testing, half the animals with each of these histories received an injection of yohimbine hydrochloride (2.0 mg/kg), an α_2-adrenoreceptor blocker intended to evoke noradrenergic system activation, while the remainder received a saline injection. Yohimbine was expected to reduce both exploration of novel stimuli and interaction with conspecifics, and it was predicted that methylphenidate would potentiate these effects. Relative to saline-tested controls, rats that received both the methylphenidate treatment and the yohimbine challenge exhibited the least exploration in the predator odor test and the lowest duration of interaction with an unfamiliar conspecific partner in the social interaction test. The behavior patterns observed in this group of rats suggest heightened emotionality and defensiveness that are typically seen when rats are administered drugs known to be anxiogenic in human subjects. In the plus-maze, exploratory locomotor activities remained unaltered by either drug while yohimbine decreased risk-assessment behaviors, an effect that remained uninfluenced by methylphenidate pretreatment.

Keywords: Methylphenidate; Yohimbine; Plus-maze; Predator odor test; Social interaction test; Defensive behavior

1. Introduction

Methylphenidate (MPD) is a commonly employed treatment for attention-deficit/hyperactivity disorder (ADHD) in both children and adults (Greenhill and Ford, 2002; Shaffer, 1994). It is a psychomotor stimulant that binds to the dopamine (DA) transporter and thereby inhibits dopamine uptake (Butcher et al., 1991; Hurd and Ungerstedt, 1989; Volkow et al., 1995). In similar fashion, it also blocks uptake of norepinephrine (NE) by its action at the NE transporter (Gatley et al., 1996). As a consequence, extracellular concentrations of both DA and NE are increased, and in this respect MPD is similar to cocaine and amphetamine (Kuczenski and Segal, 1997).

When administered repeatedly, psychomotor stimulants can result in persisting cellular and behavioural adaptations, most commonly demonstrated in mice and rats. One manifestation of this is the potentiated locomotor activity and/or stereotypy elicited by amphetamine or cocaine in animals that have a history of experience with these drugs, an effect termed behavioral sensitization (Richtand et al., 2001; Robinson and Berridge, 1993; Vanderschuren and

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Kalivas, 2000). Chronic MPD has also been found to yield such sensitization effects (Crawford et al., 1998; Eckermann et al., 2001; Gaytan et al., 1997; Gaytan et al., 2000a, b; McDougall et al., 1999; Short and Shuster, 1976; Yang et al., 2000, 2001).

In addition to an increased sensitivity to itself, chronic MPD can produce lasting changes in behavior that persist well beyond the period of drug administration in both immature and adult rodents. Bolaños et al. (2003) administered MPD at 2.0 mg/kg twice daily for 16 days to rats beginning at 20 days after birth. When tested 6 weeks after their last dose of the drug, the animals exhibited a decreased sensitivity to rewarding stimuli (sucrose, novelty, and a receptive sexual partner) and an increased sensitivity to threatening circumstances (elevated plus-maze, forced swimming, and restraint stress). Carlezon et al. (2003) administered an MPD regimen identical to that of Bolaños et al. (2003) to male rats and tested them at 60 days after birth. It was found that these animals showed more immobility and less swimming/climbing in the forced swim test, did not show a habituation of spontaneous locomotor activity during repeated exposure to an activity chamber, and were less inclined to develop a preference for a compartment associated with an injection of cocaine. Taukulis (unpublished) administered oral doses of MPD (2.5, 5.0, or 10.0 mg/kg) to young mice twice daily over a period of 1 to 6 weeks. Following a 10–12-day washout period, the animals were exposed to a natural threat: a laboratory rat, kept separated from the mouse by a wire-mesh screen. The mice treated with the two higher doses of MPD exhibited a greater avoidance of the cage nearest the rat and showed a greater frequency of jumping and hanging from the wire-mesh roof of the cage.

Psychomotor stimulant-induced neural and behavioral adaptations have generally been assumed to reflect changes in dopamine pathways, and considerable evidence has been amassed in support of this (for a review, see White and Kalivas, 1998). However, it is clear that adaptive changes can also occur in noradrenergic pathways, as studies of amphetamine have suggested (e.g., Camp et al., 1997; Harris and Williams, 1992; Herman et al., 1971; Lynch et al., 1977; Short and Shuster, 1976; Sorensen et al., 1985). Some of these studies have utilized behavioral indices indicative of a greater reactivity to threatening situations. For example, Cancela et al. (2001) found that rats treated with amphetamine for nine consecutive days subsequently exhibited a greater avoidance of the two exposed arms of a plus-maze, a behavior known to be elicited in these animals by drugs that induce anxiety in humans (e.g., Taukulis and McKay, 1992).

The primary purpose of the present study was to provide evidence of changes in noradrenergic neurotransmission resulting from chronic oral treatment with MPD. To this end, young rats were exposed to three animal models that evoke behaviors known to be modulated by the NE system: (1) an elevated plus-maze (Pellow et al., 1985b; Rodgers and Cole, 1994), (2) a predator odor (Andrews et al., 1993; Dielenberg et al., 2001), and (3) a same-sex, unfamiliar, conspecific partner (File, 1993). Half of the animals were injected with yohimbine hydrochloride (YOH), an α_2-adrenoreceptor antagonist that increases synaptic NE levels and is known to produce "anxiogenic-like" effects in various animal models that involve exposure to threatening or potentially threatening stimuli (e.g., Tanaka et al., 2000; Taukulis and McKay, 1992). YOH has been used as a challenge to assess noradrenergic hypersensitivity in human patients with panic disorder or posttraumatic stress disorder (Bremner et al., 1996; Southwick et al., 1999; Sullivan et al., 1999), in stressed macaques (Rosenblum et al., 1994), and in stressed rats (Park et al., 2001). In these studies, panic in humans and stress in animals compromised the NE system in such a manner as to produce a hyperresponsiveness to YOH. In the present experiment, it was predicted that, if chronic MPD produced lasting increases in noradrenergic sensitivity or activity, then MPD-treated rats would exhibit an increase in yohimbine-elicited, and putatively norepinephrine-mediated, behaviors. Specifically, it was hypothesized that prior MPD would potentiate any change in exploratory and interactive behaviors elicited by YOH.

A second purpose of this study was to demonstrate the utility of a new oral dosing technique for rats. Volkow and Insel (2003) have pointed out that many of the currently published studies have used intraperitoneal injections, thus delivering the drug rapidly as a concentrated bolus into an animal's system. When used clinically, MPD is delivered orally and often with food, resulting in a much slower, gradual absorption. In the present study, MPD was mixed with powdered, moistened rat chow, and the presentation procedure described herein ensured that the animals consumed their allotted portion quickly, completely, and reliably.

2. Materials and methods

2.1. Animals

Forty, 8-week-old female Long–Evans rats were used for all tests. They were housed individually in polycarbonate cages (43×21×20 cm, l×w×h) throughout the experiment. The animal holding room had a 14/10-h light/dark cycle and a temperature range of 22–24 °C. Water was available ad libitum. Food was restricted as described below. All aspects of the protocol were approved by the Animal Care Committee of the University of New Brunswick, Saint John, and adhered to the standards established by the Canadian Council on Animal Care.

Female rats were selected for this study because Blanchard et al. (1991) have shown that female rats are more anxious than male rats and consistently show greater reactivity than males on a variety of measures of defensive behaviors. Additionally, the nervous systems of female rats

may be more susceptible to alteration by psychomotor stimulants (Camp and Robinson, 1988).

2.2. Drugs

Tablets of methylphenidate (Ritalin®) were pulverized and mixed with moistened powdered rat chow ('wet mash') at a dose of 10 mg/kg/day of active drug for oral ingestion. Yohimbine hydrochloride (Sigma) was dissolved in distilled water and administered (2.0 ml/kg, i.p.) at a dose of 2.0 mg/kg 30 min prior to testing.

2.2.1. Rationale for selection of doses

In humans, the therapeutic doses of MPD typically range between 20 and 30 mg/day for adults and between 0.3 and 0.6 mg/kg/day for children (Crutchley and Temlett, 1999; Gerasimov et al., 2000). However, when using animal models, species differences must be considered when selecting comparable dosages. For instance, on a strictly mg/kg basis, rodents require higher doses than humans due to differences in metabolism (Gatley et al., 1999). That is, rats typically metabolize MPD at a faster rate than humans and doses of up to 10 mg/kg are considered low to moderate (Brandon et al., 2001; Schenk and Izenwasser, 2002).

The dose of 2.0 mg/kg of yohimbine is a low to moderate dose compared to those commonly used in the SI test (Baldwin et al., 1989; Johnston et al., 1988; Muthal and Chopde, 1994; Pellow et al., 1985a) and in the EPM (Johnston and File, 1989; Pellow et al., 1985b; Rodgers and Cole, 1994; Taukulis and McKay, 1992). To date, there are no studies that have investigated the effects of yohimbine in the POA test (Dielenberg et al., 2001). A low to moderate dose was chosen in order to avoid a strong effect of yohimbine that would prevent the detection of further potentiation of the drug's effect in the MPD pretreated group.

2.3. Apparatus

2.3.1. Elevated plus-maze (EPM) test

The elevated plus-maze (EPM) apparatus was constructed of wood and coated with black polyurethane. It had four arms set at 90° angles relative to each other. Two of the arms were exposed runways ('open' arms), surrounded only by a 1.5-cm lip to promote exploration and prevent falling. The other two arms were enclosed with 40 cm high walls on three sides. The four arms (each 50×10 cm, l×w) were joined at a central platform (10×10 cm) that had four white lines that define the entranced to each arm. Brightness was 175 lx in the open arms and 90 lx in the enclosed arms.

2.3.2. Predator odor avoidance (POA) test

The predator odor avoidance (POA) apparatus was comprised of a rectangular wood-and-glass alley with a darkened 'hiding box' at one end and a source of cat odor (a collar previously worn by a domestic cat for at least 3 weeks) suspended from a hook at the other end. The white-painted alley, measuring 120×19×32 cm (l×w×h), had a transparent (glass) front wall and a wire mesh roof. The alley was partitioned (with black lines) into three segments: a 'proximal' segment (30 cm), a center segment (30 cm), and a 'distal' segment (60 cm) that was farthest away from the collar and included the hiding box. The hiding box (30×15×30 cm, l×w×h) was made of opaque (brown) Plexiglas with an 11.5×11.5-cm opening facing the alley. Illumination (265 lx) in the areas of the alley other than the hiding box was provided by overhead fluorescent fixtures. Between testing days, the collar was stored in an airtight plastic container and kept in the freezer. Just prior to testing, it was removed from the freezer and allowed to warm. The collar was handled with rubber gloves at all times.

2.3.3. Social interaction (SI) test

The social interaction apparatus consisted of a square (60×60 cm) black-painted wooden box with 40 cm high walls and a floor partitioned into 16 squares (15×15 cm) by white lines. It was illuminated to a brightness of 50 lx.

2.4. Procedure

2.4.1. Treatment

For 10 days prior to the start of drug treatment, the animals were given 10 g of wet mash in a small porcelain dish each weekday. The wet mash consisted of one part powdered rat meal (Purina #5012) and three parts distilled water. This was supplemented with three pellets of chow (approximately 12–13 g) each evening. On weekends (Friday evening until Sunday at 1700 h), the animals had free access to chow pellets. During a subsequent 4-week treatment period, the same feeding schedule continued. However, for one half of the rats, MPD (10 mg/kg) was introduced into the wet mash. The remaining rats received unadulterated wet mash. In the post-treatment period, and for the remainder of the experiment, all of the rats were fed chow pellets ad libitum.

It is important to note that once trained all of the animals consumed 100% of the wet mash/MPD mixture within 5 min of presentation of the food. Speed of administration was therefore only slightly slower than oral administration by gavage. However, since the MPD-treated food is ingested willingly, the chronic stress attributed to the gavage procedure was avoided. Furthermore, all of the animals continued to gain weight throughout the treatment period in a steady manner consistent with the average weight gain for this strain of rats. Practically, therefore, the animals were not food-deprived but were restricted to eating at regular times each day.

2.4.2. Testing

Testing began 10 days after the last MPD treatment day. This delay was imposed based on evidence that stimulant-induced neural adaptations become more pronounced after 1

week following the discontinuation of drug administration (Laruelle, 2000). During the intervening period, the rats were gently handled and subjected to two sessions of saline injections in order to habituate the animals to these procedures. In the test phase, half of the rats (n=10) in the MPD treatment group and half the rats (n=10) in the NoMPD treatment group were injected with YOH prior to testing, while the other half (n=10) of each group received an injection of normal saline (SAL). Thus, the four experimental groups consisted of two MPD-untreated groups (NoMPD-SAL and NoMPD-YOH), and two MPD-treated groups (MPD-SAL and MPD-YOH). Immediately following the YOH or SAL injection, each rat was returned to its home cage. Thirty minutes later, the animal was transferred in its home cage from the animal holding room to the testing room.

The first part of the test phase began with the EPM test followed immediately by the POA test. The order of these tests was not counterbalanced because exposure to a cat odor can have lasting effects on subsequent activity in the EPM (Zangrossi and File, 1994), whereas studies conducted in our laboratory have found no differences in rats' behavioral responses in the POA test as a function of prior experience in the EPM. At the start of its session in the EPM, each rat was placed on the central platform, facing an enclosed arm (the enclosed arm that the rat would face was randomly selected for each rat throughout the testing period). An animal was considered to have entered an arm when all four paws had crossed the white line at the entrance to each arm. The behaviors were observed in an adjacent room via a video camera positioned above the maze. The behaviors were recorded for 5 min and later scored by a trained observer who was blind to the experimental conditions. The computer program Noldus Observer was used to tally: frequency and duration of enclosed arm entries, frequency and duration of open arm entries, and frequency of risk assessment behaviors: stretch-attend posture, and head dips. Additionally, the total open and enclosed arm entries were scored as a measure of general activity.

Immediately following the EPM test, the rats were placed in the POA apparatus. At the beginning of the POA test, the rat was placed in the 'distal' segment of the alley, facing the alley, and allowed to explore the alley freely. Five minutes later, the cat collar was placed on the hook and the rat was tested for another 5 min. The entire 10-min period was videotaped for later scoring. The behaviors of interest were: the frequency and duration of time spent in the 'proximal' segment of the alley and in the hiding box (Blanchard and Blanchard, 1989; Dielenberg et al., 2001); frequency of rearing; 'head-outs' (sitting at the entrance of the hiding box with only the head exposed); and contact with either the hook on which the cat collar was hung or the cat collar itself; and latency to first entry into the proximal segment, first rear, first head-out, and first contact with the cat collar or its hook. To control for the possible weakening of the odor stimulus over time, time of testing was counterbalanced across groups.

The SI test was conducted 20 days after the last MPD treatment. The 10-day period between this test and the previous two allowed for complete washout of yohimbine from the earlier tests and reduced the possibility of any residual effects arising from exposure to the cat odor in the POA (Zangrossi and File, 1994). This test lasted 10 min and was recorded via a video camera vertically mounted above the arena. For this test, the rats were divided into pairs, giving five pairs for each treatment condition. In addition, the experimental groups were balanced in such a manner as to ensure equal distribution with respect to drug experience in the previous tests.

Initially, the two rats were placed at opposite corners in the arena. Behaviors of interest were social interactions of both non-aggressive and aggressive types (File, 1993). The behaviors considered as non-aggressive included sniffing, following, crawling under or over each other, and passive contact. Aggressive behaviors included biting, jumping, boxing/kicking, avoiding/chasing, and fighting. Times spent exhibiting these behaviors are thought to be the most reliable indices of anxiety with decreases in duration of social interaction reflecting increases in anxiety (File, 1993). The total number of squares crossed was used as a measure of general locomotor activity (File, 1980; Guy and Gardner, 1985). Since one rat's behavior was not independent of the partner's behavior, the total duration of social interaction and total locomotor activity for each pair of rats (total of 1200 s) was used for statistical analysis (File, 1993).

In a procedure common to all three tests, the relevant test environment was cleaned immediately after an animal's trial had ended. Walls and floor were wiped with a dilute solution of isopropyl alcohol and allowed to dry before the next trial.

2.5. Statistical analysis

For each measure, a two-way (Treatment×Test) ANOVA with planned comparisons was used to evaluate the effects of MPD pretreatment and YOH challenge.

3. Results

3.1. Elevated plus-maze (EPM) test

Neither MPD pretreatment nor the YOH challenge had an effect on the percent of time that the rats spent in the open arms of the maze, the number of entries that they made into these arms, or the total number of entries they made into both types of arms (a measure of general locomotor activity). YOH was found to significantly reduce the putative 'risk assessment behaviors' of head dipping ($F(1,36)=10.96$, $P<0.05$) and stretch-attend posture ($F(1,36)=4.52$, $P<0.05$). There was no main effect of

MPD on these behaviors, nor were there any significant MPD×YOH interactions.

3.2. Predator odor avoidance (POA) test

3.2.1. Odor not present

An analysis of behavior in the POA alley during the 5 min prior to the introduction of the cat odor (Fig. 1) revealed that YOH significantly increased duration of stay in the hiding box ($F(1,36)=8.62$, $P<0.01$) and latency of first entry into the proximal segment of the alley (farthest from the hiding box) ($F(1,36)=5.61$, $P<0.05$). There was no main effect of MPD on any measure. However, an MPD×YOH interaction appeared with the following measures: duration in ($F(1,36)=4.78$, $P<0.05$) and latency of first entry into

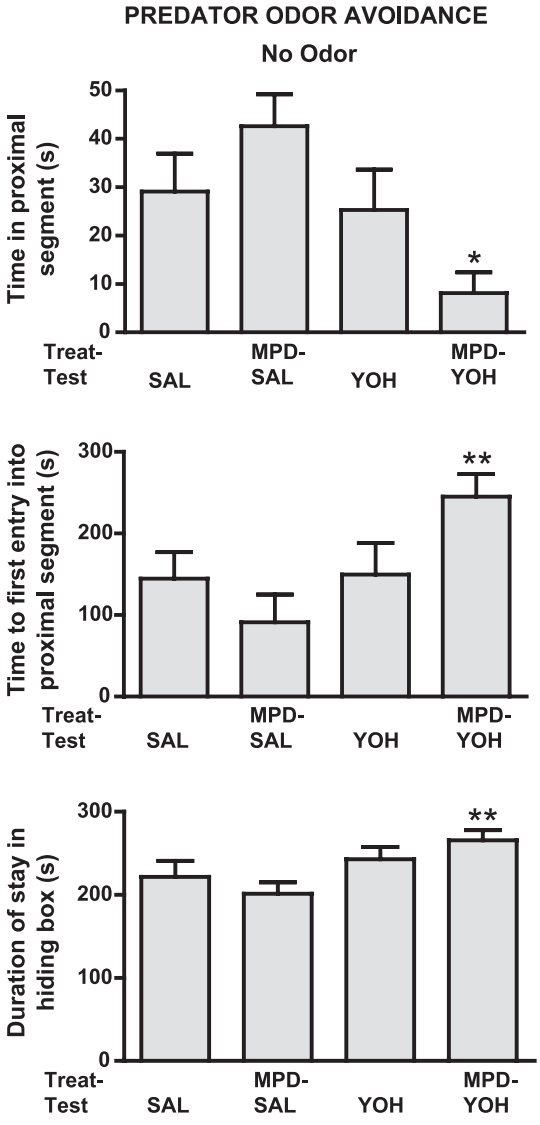

Fig. 1. Mean (±S.E.M.) time spent in the proximal segment, latency of first entry into the proximal segment, and time spent in the hiding box of the predator odor alley during the 5-min period before the cat odor was presented. Asterisks indicate a significant difference from Groups NoMPD-SAL and MPD-SAL (*$P<0.05$, **$P<0.01$).

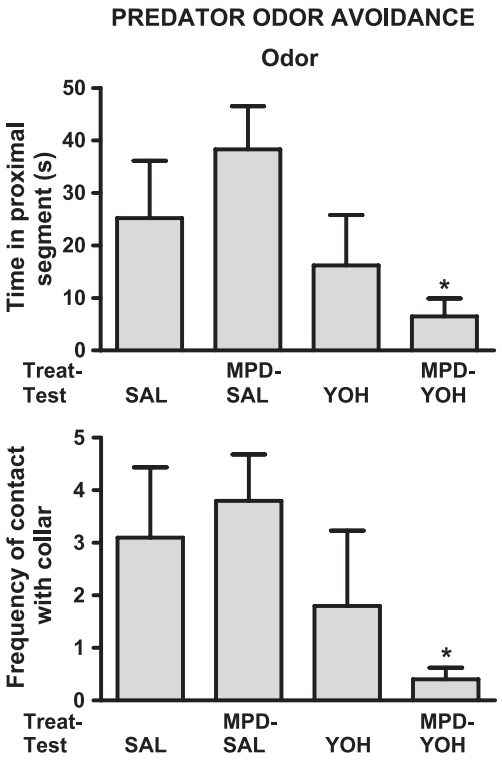

Fig. 2. Mean (±S.E.M.) time spent in the proximal segment and frequency of contacts with the cat collar during the 5-min period after the cat odor was introduced. Asterisks indicate a significant difference from Groups NoMPD-SAL and MPD-SAL (*$P<0.05$).

($F(1,36)=4.91$, $P<0.05$) the proximal segment. When planned pairwise comparisons revealed that Group NoMPD-SAL and Group MPD-SAL did not differ on any measure, linear contrasts were conducted comparing these two groups against each of the YOH-tested groups. (This was considered a more conservative test than comparing each YOH-tested group against its respective SAL-tested control because in this test, and in the SI test, Group MPD-SAL often differed from Group NoMPD-SAL, albeit nonsignificantly, in a direction that favored an MPD-SAL vs. MPD-YOH comparison over a NoMPD-SAL vs. NoMPD-YOH comparison. Assigning the two saline-tested groups equal weighting in a linear contrast against one of the YOH-tested groups reduced the influence of this factor, which tended to bias the outcome in favour of the stated hypothesis.) These revealed that only Group MPD-YOH spent less time in the segment proximal to the odor ($P<0.05$), had the greatest latency of entry into the proximal segment ($P<0.01$), and spent more time in the hiding box ($P<0.01$) than the saline-treated controls.

3.2.2. Odor present

During the 5-min period following the introduction of the cat odor (Fig. 2), a main effect of YOH was obtained for two measures: time spent in the proximal segment ($F(1,36)=5.73$, $P<0.05$) and frequency of contact made with the collar ($F(1,36)=5.64$, $P<0.05$). Linear contrasts revealed that

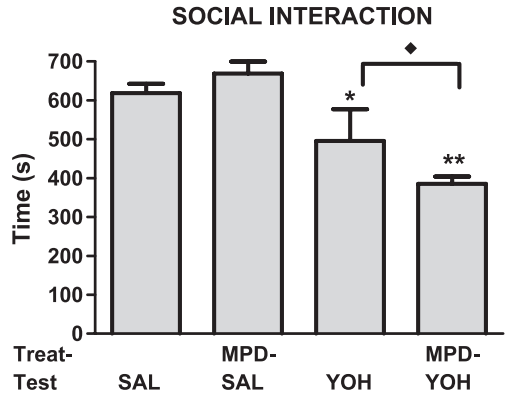

Fig. 3. Mean (±S.E.M.) time spent in social interaction. Asterisks indicate a significant difference from Groups NoMPD-SAL and MPD-SAL (*P<0.05, **P<0.001), and Group NoMPD-YOH differed from Group MPD-YOH (◆P<0.05).

MPD-YOH (but not Group NoMPD-YOH) spent less time in the segment proximal to the odor (P<0.05) and investigated the collar less frequently (P<0.05) than the saline-tested control groups.

3.3. Social interaction (SI) test

The duration of total social interaction exhibited by both rats in the SI test is shown in Fig. 3. A main effect of YOH was found (F(1,16)=22.88, P<0.001), and linear contrasts revealed that that both Group NoMPD-YOH (P<0.05) and Group MPD-YOH (P<0.001) exhibited less social interaction than the saline-tested control groups combined. No main effect of MPD emerged, but an MPD×YOH interaction was found (F(1,16)=6.04, P<0.05), which reflected the fact that rats in Group MPD-YOH interacted less than those in Group NoMPD-YOH (P<0.05).

4. Discussion

The hypothesis that a repeated, intermittent regimen of exposure to MPD would potentiate the behavioral effects of yohimbine was supported in two of the test conditions. In the predator odor avoidance (POA) and social interaction (SI) conditions, YOH engendered a greater hesitancy to explore novel stimuli and a reduced tendency to interact with a conspecific. For all of the behavioral measures taken in the POA test for which this effect of YOH reached statistical significance, the MPD-YOH group was found to differ significantly from the two saline-tested groups, whereas Group NoMPD-YOH did not. In the SI test, both these groups exhibited fewer social interactions than the saline-tested groups, but the decrease was more pronounced in Group MPD-YOH. Taken together, these findings were consistent with the contention that MPD can cause an increase in the sensitivity of neural systems that mediate affective reactions and concomitant defensive responses to mildly threatening situations.

The predator odor avoidance (POA) test is designed to confront an animal with a discrete, naturally occurring, threatening stimulus and is often conceptualized as a fear-inducing condition (Dielenberg et al., 1999). This test, in the configuration employed in the present experiment, assessed a rat's responses to both a novel environment and a cat odor. During the initial 5-min phase of the test, the group of animals that received prior MPD treatment and the YOH injection behaved as though they were fearful of leaving the hiding box or its proximity. These findings complement those of Bolaños et al. (2003) who found a decrease in locomotor activity in a novel environment shown by rats pretreated with chronic MPD, suggesting that the neural adaptations generated by the drug may have augmented the stress evoked by the situational novelty.

When the cat odor was introduced, the patterns of behavior were roughly the same, but fewer group comparisons reached significance. The reason is that activity levels in the POA were already low in the preceding 5 min; this was true for all groups, regardless of drug treatment. The presentation of the odor lowered activity in the control groups only slightly, while activity in Group MPD-YOH could not drop lower than its already minimal level. It is not known why animals of all groups were so hesitant to explore the alley. The dramatic contrast in illumination between the hiding box and the rest of the alley may have been a contributing factor. In any case, future studies using this apparatus should seek to reduce the threatening nature of the environment (or appeal of the hiding box), if the desire is to assess the rats' responses to cat odor.

The POA results complement those of Park et al. (2001) who found that chronically stressed rats exhibited a hyper-responsiveness to YOH (1.5 mg/kg). In their study, the stressors were a 5-week period of intermittent exposure to a cat and a daily change of cage-mates. In an open-field test, YOH increased immobility in both stressed and control groups, but to a substantially greater degree in the former. Park et al. (2001) suggested that their findings indicated a change in noradrenergic neurotransmission. They noted the extensive literature suggesting increased noradrenergic sensitivity of humans with panic disorder and post-traumatic stress disorder. Studies have shown that many of these patients will respond with panic attacks when they are challenged with a single dose of YOH, and long-term or severe exposure to stressors may have contributed to this hyperreactivity. In the present experiment, MPD may have behaved like a stressor in terms of its effects on noradrenergic sensitivity, and the YOH challenge may have had an effect analogous to the YOH-induced panic seen in human patients.

The social interaction (SI) test, like the POA test, is an ethologically derived model that is sensitive to manipulation by both anxiolytic and anxiogenic compounds (File, 1993; File and Seth, 2003). The rat is confronted with a conspecific partner, which elicits interactive behaviors directed towards the other animal. In the SI test conducted

in the present experiment, YOH reduced the duration of social interactions. These results are in agreement with other studies examining the effects of YOH in this model (Bhattacharya, 1995; Guy and Gardner, 1985). However, the YOH-induced reduction of this behavior was more pronounced in the group that had previously been exposed to MPD, once again implying an MPD-induced shift towards greater emotionality during periods of abnormally high noradrenergic activity.

The elevated plus-maze (EPM) is a widely used method for the evaluation of drugs with known or potential anxiolytic or anxiogenic properties. It is designed to confront animals with a novel environment in which no discrete threat is apparent. In the present study, the behavioral measures most commonly reported in investigations employing the plus-maze, the number (or percentage) of entries into and time spent in the 'open' arms of the maze remained unaffected by either MPD or YOH. The only sign of a drug effect in the EPM test was a small decline in two putative risk-assessment behaviors, head-dips and stretch-attend posture, elicited by YOH irrespective of previous experience with MPD. It is not uncommon for risk assessment measures to be more sensitive to drug manipulation than the open-arm exploration indices (Rodgers and Cole, 1994), and here they revealed that YOH was not without behavioral effect. But unlike the POA and SI tests, a prior history of MPD did not yield a potentiated response. The minimal effect of YOH in this context was puzzling, given previous evidence that exploratory behaviors in the plus-maze can be sensitive to this compound (e.g., Taukulis and McKay, 1992). However, it is possible that the gender of the rats may influence outcomes in this test. In the present experiment, female rats were used, whereas most studies of yohimbine in the EPM have tested males. It is also true that the results reported for YOH in this maze have been inconsistent (Rodgers and Cole, 1994), and therefore it may not be a reliable method for assessing this drug.

The behavior patterns observed in Group MPD-YOH in the POA and SI tests suggested heightened emotionality and defensiveness. These behavioral outcomes were typical of those patterns seen when rats are administered drugs known to be anxiogenic in human subjects. The noradrenergic cells of the locus coeruleus (LC) are known to play a contributory role in the manifestation of these behaviors. In particular, the LC serves as a modulator of the intensity of the response its target sites, such as the hippocampus, amygdala, and prefrontal cortex, show to their afferent inputs (Aston-Jones et al., 1999; Berridge and Waterhouse, 2003; Charney et al., 1995). The behavioral responses coordinated by the target regions will differ depending upon the amplitude of coerulear priming. YOH is presumed to potentiate this priming by blocking α_2-adrenoreceptors located on the presynaptic membranes of coerulear neurons, thus assuring higher levels of synaptic NE.

Chronic MPD exposure may modulate YOH's effects in one of several ways. For instance, MPD is known to increase extracellular NE, probably by inhibiting NE uptake. These chronically elevated levels of NE may yield alterations in the presynaptic or postsynaptic cell that enhance the impact of subsequent releases of this neurotransmitter (Harris and Williams, 1992). In this regard, MPD may mimic the effects of stressors, which also increase NE levels by increasing the synthesis, release, and catabolism of NE in LC neurons, and which will also, as noted by Park et al. (2001), potentiate YOH. As Zigmond et al. (1995) delineated, there are numerous ways in which this may come about. One possibility is that stressors and perhaps MPD as well, may alter the sensitivity of α_2 receptors. This may have important implications for clinicians when considering options for treatment of ADHD patients with co-morbid anxiety particularly with the advent of new medications for ADHD, such as atomoxetine, that exclusively target the NE system.

The failure to detect an effect of MPD alone (Group MPD-SAL) in these tests was surprising in light of reports that this drug can produce behavioral sensitization in rats and mice (e.g., Crawford et al., 1998; Gaytan et al., 1997; McDougall et al., 1999). Several factors may account for this discrepancy. In most other studies, the MPD has been injected, not delivered orally; and the behavioral measures taken have been general locomotion in an open field or stereotypy, both believed to be indices of dopaminergic sensitivity. In the only study that also used oral delivery as the method of administration and measured behavioral reactivity to a predator without further drug challenge (Taukulis, unpublished), MPD doses were administered twice per day to mice. Potential species differences aside, the neural effects of the single daily dose delivered orally to the rats in the present experiment may have yielded an effect that was too weak to be detected except when an animal was subjected to an additional activation of its noradrenergic systems.

This experiment introduced an oral delivery method through which MPD (and perhaps other drugs) may be administered easily and reliably. It is important to study the impact of drugs administered in this fashion because the pharmacokinetics of the drug will be substantially different when compared with IP injection. An oral dose is absorbed more slowly and has a potentially milder impact on its target regions due to lower extracellular concentrations (Gerasimov et al., 2000; Kuczenski and Segal, 2002). The simultaneous presence of food in the stomach is likely to further slow absorption. Unpublished findings in this laboratory have found dose-related elevations of locomotor activity following MPD ingested with wet mash; this effect appeared shortly after ingestion and persisted undiminished for more than 60 min thereafter. Response–time curves were flat across this interval for all doses. Other studies have delivered oral MPD doses by gavage through an intragastric cannula implanted surgically. The present procedure is simpler to implement and avoids the stress of the surgical procedure. Addition-

ally, the simultaneous presence of food in the stomach more closely mimics the usual conditions under which the drug is delivered to human patients for therapeutic purposes.

Kuczenski and Segal (2002) and others have argued that their evidence suggests that potential methylphenidate sensitization is not a concern for human recipients of the drug because such an effect is unlikely to occur at clinically relevant doses when these are delivered orally. However, these same authors reported that doses that did not increase locomotion (and, in fact, slightly decreased it) created elevated levels of NE in the hippocampus while leaving dopamine levels in the nucleus accumbens unchanged. Locomotor activity thus seems to be an insensitive measure of NE-based alteration due to chronic stimulant exposure. More elaborate methods, such as those employed in the present experiment, may be necessary to detect the phenomenon via behavioral means.

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References

Andrews N, Barnes NM, Steward LJ, West KE, Cunningham J, Wu P-Y, et al. A comparison of rat brain amino acid and monamine content in diazepam withdrawal and after exposure to a phobic stimulus. Br J Pharmacol 1993;109:171–4.

Aston-Jones G, Rajkowski J, Cohen J. Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry 1999;46: 1309–20.

Baldwin HA, Johnston AL, File SE. Antagonistic effects of caffeine and yohimbine in animal tests of anxiety. Eur J Pharmacol 1989; 159:211–5.

Berridge CW, Waterhouse BD. The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev 2003;42:33–84.

Bhattacharya SK. Anxiogenic activity of centrally administered scorpion (*Mesobuthus tamulus*) venom in rats. Toxicon 1995;33:1491–9.

Blanchard RJ, Blanchard DC. Attack and defense in rodents as ethoexperimental models for the study of emotion. Prog Neuro-Psychopharmacol Biol Psychiatry 1989;13:3–14.

Blanchard DC, Shepherd JK, De Padua Carobrez A, Blanchard RJ. Sex effects in defensive behavior: Baseline differences and drug interactions. Neurosci Biobehav Rev 1991;15:461–8.

Bolaños CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. Biol Psychiatry 2003;54:1317–29.

Brandon CL, Marinelli M, Baker LK, White FJ. Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. Neuropsychopharmacology 2001;25:651–61.

Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: II. Clinical studies. Synapse 1996; 23:39–51.

Butcher SP, Liptrot J, Aburthnott GW. Characterisation of methylphenidate and nomifensine induced dopamine release in the rat striatum using in vivo brain microdialysis. Neurosci Lett 1991;122:245–8.

Camp DM, Robinson TE. Susceptibility to sensitisation: II. The influence of gonadal hormones on the enduring changes in brain monamines and behavior produced by the repeated administration of D-amphetamine or restraint stress. Behav Brain Res 1988;30:69–88.

Camp DM, DeJonghe DK, Robinson TE. Time-dependent effects of repeated amphetamine treatment on norepinephrine in the hypothalamus and hippocampus assessed with in vivo microdialysis. Neuropsychopharmacology 1997;17:130–40.

Cancela LM, Basso AM, Martijena ID, Capriles NR, Molina VA. A dopaminergic mechanism is involved in the "anxiogenic-like" response induced by chronic amphetamine treatment: a behavioral and neurochemical study. Brain Res 2001;909:179–86.

Carlezon WA, Mague Jr SD, Anderson SL. Enduring behavioural effects of early exposure to methylphenidate in rats. Biol Psychiatry 2003; 54:1330–7.

Charney DS, Deutch AY, Southwick SM, Krystal JH. Neural circuits and mechanisms of post-traumatic stress disorder. In: Friedman MJ, Charney DS, Deutch AY, editors. Neurobiological and clinical consequences of stress: from normal adaptations to PTSD. Philadelphia: Lippincott-Raven; 1995. p. 271–87.

Crawford CA, McDougall SA, Meier TL, Collins RL, Watson JB. Repeated methylphenidate treatment induces behavioral sensitization and decreases protein kinase A and dopamine-stimulated adenylyl cyclase activity in the dorsal striatum. Psychopharmacology 1998; 136:34–43.

Crutchley A, Temlett JA. Methylphenidate (Ritalin) use and abuse. S Afr Med J 1999;89:1076–9.

Dielenberg RA, Arnold JC, McGregor IS. Low-dose midazolam attenuates predatory odor avoidance in rats. Pharmacol Biochem Behav 1999;2: 197–201.

Dielenberg RA, Hunt GE, McGregor IS. "When a rat smells a cat": the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. Neuroscience 2001;104:1085–97.

Eckermann K, Beasley A, Yang P, Gaytan O, Swann A, Dafny N. Methylphenidate sensitization is modulated by valproate. Life Sci 2001;69:47–57.

File SE. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J Neurosci Methods 1980;2: 219–38.

File SE. The social interaction test of anxiety. Neurosci Prot 1993;10:1–7.

File SE, Seth P. A review of 25 years of the social interaction test. Eur J Pharmacol 2003;463:35–53.

Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. Life Sci 1996;58:231–9.

Gatley SJ, Volkow ND, Gifford AN, Fowler JS, Dewey SL, Ding Y-S, et al. Dopamine-transporter occupancy after intravenous doses of cocaine and methylphenidate in mice and humans. Psychopharmacology 1999;146: 93–100.

Gaytan O, al-Rahim S, Swann A, Dafny N. Sensitization to the locomotor effects of methylphenidate in the rat. Life Sci 1997;61:101–7.

Gaytan O, Nason R, Alagugurusamy R, Swann A, Dafny N. MK-801 blocks the development of sensitization to the locomotor effects of methylphenidate. Brain Res Bull 2000a;51:485–92.

Gaytan O, Yang P, Swann A, Dafny N. Diurnal differences in sensitization to methylphenidate. Brain Res 2000b;864:24–39.

Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, et al. Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. J Pharmacol Exp Ther 2000;295:51–7.

Greenhill LL, Ford RE. Childhood attention-deficit hyperactivity disorder: pharmacological treatments. In: Nathan PE, Gorman JM, editors. A guide to treatments that work, 2nd ed. New York: Oxford University Press; 2002. p. 25–55.

Guy AP, Gardner CR. Pharmacological characterisation of a modified social interaction model of anxiety in the rat. Neuropsychobiology 1985;14: 194–200.

Harris GC, Williams JT. Sensitization of locus coeruleus neurons during withdrawal from chronic stimulants and antidepressants. J Pharmacol Exp Ther 1992;261:476–83.

Herman ZS, Trzeciak H, Chrusciel TL, Kmieciak KK, Drybanski A, Sokola A. The influence of prolonged amphetamine treatment and amphetamine withdrawal on brain biogenic amine content and behaviour in the rat. Psychopharmacologia 1971;21:74–81.

Hurd YL, Ungerstedt U. In vivo neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate–putamen. Eur J Pharmacol 1989;166:251–60.

Johnston AL, File SE. Yohimbine's anxiogenic action: evidence for noradrenergic and dopaminergic sites. Pharmacol Biochem Behav 1989;32:151–6Johnston A.L., Baldwin H.A., File S.E.. Measures of anxiety and stress in the rat following chronic treatment with yohimbine. J Psychopharmacol 1988;2:33–8.

Johnston AL, Baldwin HA, File SE. Measures of anxiety and stress in the rat following chronic treatment with yohimbine. J Psychopharmacol 1988;2:33–8.

Kuczenski R, Segal DS. Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. J Neurochem 1997;68:2032–7.

Kuczenski R, Segal DS. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. J Neurosci 2002;22:7264–71.

Laruelle M. The role of endogenous sensitization in the pathology of schizophrenia: implications from brain imaging studies. Brain Res Rev 2000;31:371–84.

Lynch M, Kenny M, Leonard BE. The effect of chronic administration of D-amphetamine on regional changes in catecholamines in the rat brain. J Neurosci Res 1977;3:295–300.

McDougall SA, Collins RL, Karper PE, Watson JB, Crawford CA. Effects of repeated methylphenidate treatment in the young rat: sensitization of both locomotor activity and stereotyped sniffing. Exp Clin Psychopharmacol 1999;7:208–18.

Muthal AV, Chopde CT. Anxiolytic effect of neuropeptide FMRFamide in rats. Neuropeptides 1994;27:105–8.

Park CR, Campbell AM, Diamond DM. Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in adult rats. Biol Psychiatry 2001;50:994–1004.

Pellow S, Chopin P, File SE. Are the anxiogenic effects of yohimbine mediated by its action at benzodiazepine receptors? Neurosci Lett 1985a;55:5–9.

Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985b;14:149–67.

Richtand NM, Woods SC, Berger SP, Strakowski SM. D3 dopamine receptor, behavioral sensitization, and psychosis. Neurosci Biobehav Rev 2001;25:427–43.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 1993;18:247–91.

Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology, and ethology. In: Cooper SJ, Hendrie CA, editors. Ethology and psychopharmacology. New York: Wiley; 1994. p. 9–44.

Rosenblum LA, Coplan JD, Friedman S, Bassoff JM, Gorman JM, Andrew NW. Adverse early experiences affect noradrenergic and serotonergic functioning in adult primates. Biol Psychiatry 1994;35:221–7.

Schenk S, Izenwasser S. Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine. Pharmacol Biochem Behav 2002; 72:651–7.

Shaffer D. Attention deficit hyperactivity disorder in adults. Am J Psychiatry 1994;151:633–8.

Short PH, Shuster L. Changes in brain norepinephrine associated with sensitization to D-amphetamine. Psychopharmacology 1976;48:59–67.

Sorensen SM, Hattox S, Johnson SW, Bickford P, Murphy R, Freedman R. Norepinephrine-dependent and independent mechanisms of persistent effects of amphetamine in rat cerebellum. Life Sci 1985;36: 2383–9.

Southwick SM, Morgan III CA, Charney DS, High JR. Yohimbine use in a natural setting: effects on posttraumatic stress disorder. Biol Psychiatry 1999;46:442–4.

Sullivan GM, Coplan JD, Kent JM, Gorman JM. The noradrenergic system in pathological anxiety: a focus on panic with relevance to generalized anxiety and phobias. Biol Psychiatry 1999;46:1205–18.

Tanaka M, Yoshida M, Emoto H, Ishii H. Noradrenaline systems in the hypothalamus, amygdala, and locus coeruleus are involved in the provocation of anxiety: basic studies. Eur J Pharmacol 2000;405: 397–406.

Taukulis HK, McKay RW. Postdrug retention of diazepam's effects on habituation to a novel environment in an animal model of anxiety. Psychobiology 1992;20:286–93.

Vanderschuren LJMJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 2000;151:99–120.

Volkow ND, Insel TR. What are the long-term effects of methylphenidate treatment? Biol Psychiatry 2003;54:1307–9.

Volkow ND, Ding Y-U, Fowler JS, Wang G-J, Logan J, Gatley JS, et al. Is methylphenidate like cocaine? Studies on their pharmacokinetics and distribution in the human brain. Arch Gen Psychiatry 1995;52:456–63.

White FJ, Kalivas PW. Neuroadaptations involved in amphetamine and cocaine addiction. Drug Alcohol Depend 1998;51:141–53.

Yang P, Beasley A, Eckermann K, Swann A, Dafny N. Valproate prevents the induction of sensitization to methylphenidate (Ritalin) in rats. Brain Res 2000;887:276–84.

Yang P, Singhal N, Modi G, Swann A, Dafny N. Effects of lithium chloride on induction and expression of methylphenidate sensitization. Eur J Pharmacol 2001;426:65–72.

Zangrossi Jr H, File SE. Habituation and generalization of phobic responses to cat odor. Brain Res Bull 1994;33:189–94.

Zigmond MJ, Finlay JM, Sved AF. Neurochemical studies of central noradrenergic responses to acute and chronic stress. In: Friedman MJ, Charney DS, Deutch AY, editors. Neurobiological and clinical consequences of stress: from normal adaptations to PTSD. Philadelphia: Lippincott-Raven; 1995. p. 45–60.