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Stereotyped Behavior: Effects of *d*-Amphetamine and Methylphenidate in the Young Rat

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ROFFMAN, J. L. AND L. A. RASKIN. *Stereotyped behavior: Effects of* d-*Amphetamine and methylphenidate in the young rat*. PHARMACOL BIOCHEM BEHAV **58**(4) 1095–1102, 1997.—The proclivity of *d*-amphetamine and methylphenidate to induce perseverative motoric and vocal side effects detracts from the clinical efficacy of these stimulants in the treatment of Attention-Deficit Hyperactivity Disorder (ADHD). In an attempt to develop a model for these deleterious treatment effects, this study explored the behavioral influences exerted by *d*-amphetamine and methylphenidate in the young laboratory rat. This experiment revealed that doses of these stimulants that typically induce stereotypy provoke diverging behavioral profiles: while animals given 5 mg/kg *d*-amphetamine exhibited repetitive sniffing activity, rats treated with 30 mg/kg methyl-phenidate displayed perseverative gnawing behaviors. Although pretreatment with the serotonin synthesis inhibitor *p*-chlorophenylalanine (PCPA) significantly attenuated both stimulant-induced stereotypies, the effect of PCPA on *d*-amphetamine-induced sniffing was more profound than on methylphenidate-induced gnawing. High-performance liquid chromatography (HPLC) analysis of monoamine levels in the striatum, frontal cortex, and thalamus indicated that PCPA induced an overall 89% depletion of serotonin across all conditions. These findings shed some light on the neurochemical mechanisms that underlie the differential effects of *d*-amphetamine and methylphenidate on stereotyped motor activity in the rat, and suggest future experiments for understanding the role of serotonin in such effects. Further, these results have implications for the differential side effects observed from each of these stimulants when used clinically in children with ADHD. © 1997 Elsevier Science. Inc.

Stereotyped behavior *d*-Amphetamine Methylphenidate Rats

d-AMPHETAMINE (DEX) and methylphenidate (MPH) produce dose-dependent stereotypy in the rat. In a pioneering study Fog (11) observed the onset of stereotyped sniffing, licking, and biting after treatment with DEX or MPH. These motor stereotypies, which lasted about an hour with DEX (10 mg/kg) and several hours with MPH (100 mg/kg), were decoupled from exploratory behavior, forward locomotion, and grooming, a phenomenon elaborated upon by Robbins and Sahakian (28). Randrup and Munkvad (26) described a dose-dependent spectrum of DEX-induced motor activities, while Costall and Naylor (5) found that high doses of MPH-induced stereotypy exhibited a dose-dependent intensity. These findings are consistent with the Lyon and Robbins hypothesis (20), which suggests that increased doses of DEX and other stimulants will result simultaneously in an increased intensity of a focused behavioral response and a decrease in the number of active response categories.

Interestingly, data from Pechnick, Janowsky and Judd (25) suggests a dissociation between the effects of MPH and DEX at high doses. At drug concentrations equal to or greater than 111 μmol (30 mg/kg), MPH-treated rats scored significantly higher in stereotypy ratings (mean score = 3.0) than rats treated with equimolar concentrations of DEX (mean score = 2.3). Given the 0–4 point stereotypy scale employed by Pechnick et al., where a score of "0" indicated behavior similar to saline-treated rats, "2" indicated continuous sniffing behavior, and "4" indicated continuous gnawing behavior, these results indicated that while MPH induced repetitive gnawing at high doses, acute DEX treatment tended to produce stereotyped sniffing activity. Several studies have verified the existence of these and other differential effects of these two stimulants. Among his initial observations, Fog (11) reported more biting after 100 mg/kg MPH than after 10 mg/kg DEX. In his review, Kuczenski (16) established that DEX is approximately 10

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times more potent than MPH in producing intense stereotypy; thus, Fog's observation cannot be explained by the difference in dosages utilized. Moreover, Mueller (23) found that moderate doses of DEX induced more obvious, consistent locomotor stereotypy (as indicated by highly consistent path-retracing behavior) than comparable doses of MPH. Finally, Fray et al. (12) reported that even at very high doses (15 mg/kg), DEX rarely induced licking and gnawing behaviors similar to those observed by Pechnick et al. (25) following high doses of MPH.

It is also noteworthy that in clinical situations investigators have reported that chronic DEX and MPH therapy can induce diverging compulsive side effects in children undergoing psychostimulant treatment for ADHD. In a double-blind study of 45 hyperactive boys, Borcherding et al. (3) found that 34 children exhibited stereotyped orofacial movements, compulsive hand motions, repetitive eye blinking or head jerking, perserverative grunting, or tremor following stimulant therapy; behaviors during treatment were compared to a 2-week medication-free baseline period. These effects were not related to clinical improvement. DEX- and MPH-treated boys demonstrated equal proclivities towards orofacial and other head-related stereotypies, as well as repetitive hand motions. However, more so than with MPH treatment, amphetamine tended to increase cleaning and checking behaviors resembling childhood-onset Obsessive-Compulsive Disorder (OCD); one DEX-treated child became uncharacteristically compulsive about keeping his room clean, another began methodically buttoning and then folding laundry, and a third raked leaves for 7 consecutive hours. Conversely, MPH-treated children tended to display perfectionistic, detail-oriented behavior, such as rewriting work or coloring over and over the same area. Children in the MPH condition also exhibited a significant tendency to display simultaneously abnormal movements and compulsive behavior. These differential side effects suggest the possibility that MPH and DEX exert their effects in part through separate neural pathways; in particular, the Borcherding study suggests an association of DEX and not MPH with classical OCD symptoms and, therefore, serotonergic mechanisms.

The subtle differences produced in stereotyped behavior by DEX and MPH in laboratory animals (25) and the reported differences in motor side effects of these stimulant reported in clinical populations (3) provide the conceptual foundation for this study. The purpose here is twofold. First, to extend the observations of Pechnicket et al., we examined the qualitative difference in stereotypies induced by DEX and MPH in young rats, employing a more appropriate model for childhood disorders by taking advantage of the comparable immaturity of the CNS in both species [see (21) for a review]. Working from the assumption that repetitive gnawing does not necessarily represent a more stereotyped activity than repetitive sniffing (11), this experiment attempted to evaluate stimulant-induced stereotypy, not in light of a predefined continuum of behaviors (25) but rather in terms of the frequency and duration of a range of perseverative activities over each recording period (1). This approach seemed more consistent with the wide range of stimulant-induced motor effects observed in children receiving treatment for ADHD (3). Second, given the apparent modulatory effects of striatal and cortical 5-HT on motor stereotypies in the rat (17,27), as well as the widely reported connection between 5-HT and OCD (10,13,32,33), we investigated the role of serotonin in the elicitation of the observed stereotyped behaviors by examining the effects of pretreatment with *p*-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor, on DEX- and MPH-induced stereotypies.

METHOD

Subjects

Experimentally naive male Sprague–Dawley rats (Charles River Laboratories), 75 to 100 g and 4-weeks-old at the time of purchase, were housed individually in cages and given free access to food and water. Animals were not utilized until at least 48 h after their arrival to permit acclimation to a reverse, 24 h light–dark cycle.

Apparatus

Behavioral testing occurred in a black room with two overhead red lights. Two rats at a time were placed individually in 20-gallon glass aquariums containing wood shavings. Two video cameras were placed on opposite sides of the cages at a distance that permitted recording from both aquariums simultaneously. Wood shavings were changed after each testing session; at the end of the testing day, the aquariums were wiped down with Simple Green all-purpose cleaner (Sunshine Makers).

Procedure

Drugs. The two psychostimulants used in Experiment I included *d*-amphetamine sulfate (DEX) and methylphenidate hydrochloride (MPH). Some animals were also pretreated with *p*-chlorophenylalanine methyl ester hydrochloride (PCPA). All drugs were obtained from Sigma. Drugs were dissolved at room temperature in a vehicle solution of 0.9% isotonic saline and were administered intraperitoneally. Solutions were freshly prepared every 1–2 days and were injected at a volume of 0.1 ml per 100 g body weight.

Dosage. Given the well-documented rate-dependent effects of psychostimulants [see (6) and (20) for reviews], we selected doses of DEX and MPH that would be equally effective in inducing stereotypy. Experimenters have consistently found that 5 mg/kg DEX induces stereotyped sniffing in the absence of locomotion (7,18,25,30); Kuczenski et al. (17) further reported that 5 mg/kg DEX provoked a multiphasic behavioral response, including (but not limited to) a stereotypy phase consisting of repetitive oral, sniffing, and head-swinging activities. Because the 5 mg/kg dose of DEX is high enough to induce stereotyped sniffing, but low enough to foster a variety of other drug-related behaviors, this dose was selected for the present experiment.

For similar reasons, this study employed a MPH dose of 30 mg/kg. Costall and Naylor (5) noted that while MPH concentrations ranging from 30 mg/kg to 40 mg/kg induced the same amount of stereotyped gnawing, the 40 mg/kg dose resulted in a reduced overall behavioral profile; conversely, as with 5 mg/kg DEX, several experimenters have found 30 mg/kg MPH to induce a multiphasic pattern of activity including a period of focused motor stereotypy (17,22,25).

The observations of Pechnick et al. (25) also suggest that 30 mg/kg MPH presents an appropriate dose for comparing the effects of that drug to DEX. Below 30 mg/kg MPH, rats exhibited the same degree of stereotyped sniffing as those given equimolor concentrations of DEX; however, above 30 mg/kg, MPH-treated animals demonstrated substantially less sniffing than DEX-treated rats. Furthermore, at MPH doses of 30 mg/kg and above, a higher percentage of animals exhibited stereotyped gnawing than those given equimolar (or greater) doses of DEX. In light of these findings, 5 mg/kg DEX and 30 mg/kg MPH appear to represent equipotent

doses vis-a-vis the Lyon and Robbins hypothesis (20): these doses promote similar degrees of focused stereotypy at the expense of a variety of other response categories.

With regard to PCPA, peak serotonin depletion has been found to occur 48 h following initial treatment (15); moreover, Segal (30) reported that 48 h following PCPA injection (300 mg/kg), DEX-treated animals exhibited increased crossover and rearing activity, and decreased focused motor stereotypies, relative to DEX-injected animals given no PCPA pretreatment. Dickinson and Curzon (7) observed that pretreatment with 180 mg/kg PCPA 48 and 24 h prior to DEX injection resulted in an 82% reduction in central 5-HT concentration; furthermore, this pretreatment schedule resulted in the significant attenuation of DEX-induced sniffing. Accordingly, the PCPA treatment method employed by Dickinson and Curzon served as a model for the present experiment.

Testing. Animals were divided into two large groups; one group was used for assays, and the other for behavioral testing. Stereotypy was elicited following drug injection before all biochemical analyses were performed. Half of the animals received pretreatments of PCPA (180 mg/kg) 48 and 24 h in advance of behavioral testing; the other half received comparable saline pretreatments (SAL). Within each of these samples animals were further divided into three groups: on testing days, one group received 5 mg/kg DEX, a second received 30 mg/kg MPH, and a third received saline (SAL). The number of animals in each behavioral testing group were as follows, with drug condition listed as "pretreatment/treatment": SAL/SAL, $n = 6$; PCPA/SAL, $n = 6$; SAL/DEX, $n = 6$; PCPA/DEX, $n = 6$; SAL/MPH, $n = 5$; PCPA/MPH, $n = 5$.

Testing occurred during the dark phase of the reverse light–dark cycle. Animals were removed from their home cage and placed in the testing apparatus for 10 min to permit habituation. Animals were then weighted and injected by drug condition; following the injection, the rats were placed again in the testing apparatus and the aquariums were positioned in the black room with red lighting. Video recording commenced 10 min after injection and concluded 2 h later.

Scoring and behavioral ratings. Video tapes were examined by a trained observer who was blind to the pretreatment and treatment conditions of the animals. To gain a representative sampling of behaviors, the observer recorded activity during the last 2 min of every successive 10-min interval, beginning at 18–20 min postinjection and ending at 128–130 min postinjection. During each 2-min interval, the duration of each of the following activities was recorded if they occurred at moderate to high intensity for 3 or more seconds without concurrent locomotion: lying still, sniffing chips or air, scratching, head swinging, gnawing at chips or apparatus, extended rearing, repeated rearing, moving rear, and grooming.

Unlike the stereotypy scale employed by Pechnick et al. (25), which rated perseverative gnawing as "more stereotyped" than repetitive sniffing, this method of behavioral recording, derived from the work of Russell and Pihl (29) and Antoniou and Kafetzopoulos (1), affords equal consideration to a range of stereotyped behaviors. While Pechnick et al. did not attempt to substantiate their hierarchical rating system, the present recording method reflects those of several experimenters who consider perseverative sniffing and gnawing to be motor behaviors of equal stereotypic character (1,11,29). Furthermore, this method allowed us to measure the average duration of each episode as well as the frequency of each perseverative behavior, enabling us to characterize the observed behaviors as "stereotyped" if they differed from those seen in control animals along both criteria.

High performance liquid chromatography. Brain dissection followed a modified protocol of Chung et al. (4). Brains were removed rapidly and frontal cortex, striatal, and thalamic tissues, localized with the aid of a stereotaxic atlas (24), were dissected in that order from three millimeter-thick coronal slices and immediately frozen over dry ice. Tissues from both sides of the brain were combined for analysis. Frozen tissues were weighed, placed in centrifuge tubes, and homogenized in 0.2 M perchloric acid (ESA). Homogenized samples were spun in a refrigerated centrifuge at 18,000 revolutions per second for ten minutes and supernatant was injected into the HPLC system (specifications given below). Serotonin peaks were identified and sample 5-HT content was determined by comparison to external standards (Sigma).

The HPLC system and conditions used were as follows: (a) HPLC and detector (ESA): Coulochem II System with Coulochem Organizer Module, Solvent Delivery module (model 580), Guard Cell (model 5020), and Analytical Cell (model 5011); (b) column (Keystone Scientific): pore size = 120 Å, particle size = 3 μm, dimensions = 150 × 4.6 mm; (c) mobile phase (ESA): 75 mM sodium dihydrogen phosphate, monohydrate; 1.7 mM 1-octanesulfonic acid, sodium salt; 25 μm EDTA; 10% (vol/vol) acetonitrile, HPLC grade; pH = 3.00; (d) guard cell potential: 375 mV; (e) analytical cell potential: −60 mV; (f) gain: 200 nA.

Statistical testing. A 2 × 3 ANOVA was used to determine the effects of drug pretreatment and treatment on the various behaviors examined, as well as to test for pretreatment × treatment interactions. Subsequent one-way ANOVA collapsed across pretreatment conditions assessed significant differences between treatment groups; post hoc Tukey multiple comparisons testing localized these differences to particular treatment groups. If the interaction from the two-way ANOVA reached significance, post hoc Tukey HSD tests were performed to determine the effect of PCPA pretreatment on treatment-induced behaviors.

Average duration of behavioral episodes as a function of pretreatment and time was determined for a given treatment group by performing a 2 × 12 ANOVA using data from each of the 12 observation periods. If a significant pretreatment × time interaction was obtained, a post hoc Tukey HSD test was conducted to compare baseline and peak behavior intensity among SAL and PCPA-pretreated animals.

The effects of drug pretreatment and treatment on 5-HT levels in the striatum, frontal cortex, and thalamus were examined using a 2 × 3 ANOVA. For each brain area, a Student's *t*-test was conducted to assess whether PCPA similarly depleted 5-HT across animals in the DEX and MPH treatment conditions.

RESULTS

Low-Frequency Behaviors

Although nine behaviors were examined in this study, several were not analyzed due to an insufficient number of occurrences in any treatment condition. While animals receiving SAL treatment on the test day exhibited more grooming than animals in the DEX or MPH treatment conditions, even those animals in the SAL condition demonstrated this behavior for an average of only 30.3 s during the entire range of observation periods (2400 s). Similarly, rearing behaviors were seldom observed in any treatment condition after the first few observation periods; scratching and head swinging behaviors were seen even less frequently.

Overall Effects of DEX and MPH

d-Amphetamine and methylphenidate exerted dramatic effects on overall activity (Table 1). A comparison of total time spent lying still across all pretreatment and treatment conditions was attempted through a preliminary 2 × 3 ANOVA, which indicated a highly significant treatment effect, $F(2, 28) = 45.41$, $p < 0.005$; pretreatment effects did not reach significance. Subsequent one-way ANOVA collapsed across pretreatment conditions indicated a highly significant difference among treatment groups, $F(2, 31) = 49.4$, $p < 0.005$; a post hoc Tukey multiple comparisons test revealed that animals in the saline treatment condition spent significantly more time lying still than stimulant-treated animals ($p < 0.05$).

DEX-Induced Stereotypy

DEX exerted a profound effect upon sniffing behavior (Table 1). A 2 × 3 ANOVA indicated a highly significant main treatment effect, $F(2, 28) = 23.3$, $p < 0.005$, a significant main pretreatment effect, $F(1, 28) = 4.3$, $p < 0.05$, and a significant pretreatment × treatment interaction, $F(2, 28) = 5.2$, $p < 0.05$. As collapsed across treatment conditions, mean sniffing scores among SAL pretreated animals were higher than those among PCPA pretreated animals. One-way ANOVA indicated a highly significant difference among the three treatment groups, as collapsed across pretreatment conditions, $F(2, 31) = 16.9$, $p < 0.005$; a post hoc Tukey multiple comparisons test revealed that animals in the DEX treatment condition exhibited significantly more sniffing than those in the SAL or MPH treatment conditions.

However, because the pretreatment × treatment interaction in the two-way ANOVA reached significance, a post hoc Tukey HSD test was performed to compare group means within the same level of each independent variable. This analysis revealed that animals in the SAL/DEX condition exhibited significantly more sniffing than rats in the SAL/SAL, SAL/MPH, or PCPA/DEX conditions ($p < 0.05$). No significant differences were found between SAL- and PCPA-pretreated groups in the SAL test-day treatment condition, indicating that by itself PCPA did not affect the expression of sniffing behaviors. Similarly, MPH by itself did not exert a large effect on sniffing; animals in the SAL/MPH condition exhibited lower total sniffing than animals in the SAL/SAL condition, but this difference did not reach significance. Finally, there existed no significant differences among any of the three groups receiving PCPA pretreatment, although rats in the PCPA/DEX condition expressed a slightly greater degree of sniffing behavior. In summary, while DEX dramatically potentiated total sniffing behavior, PCPA pretreatment depressed DEX-induces sniffing to the level of all SAL- and MPH-treated animals.

To further evaluate the effect of PCPA on DEX-provoked sniffing, we determined average duration per sniffing episode as a function of time for rats in the SAL/DEX and PCPA/DEX conditions (Fig. 1). Examining first the data from SAL-pretreated animals, it is clear that DEX induces a multiphasic behavioral response: sniffing duration became increasingly prolonged over time, peaking 70–80 min after DEX injection, and then declined to initial intensity. However, animals in the PCPA pretreatment condition exhibited a behavioral pattern of considerably lower variability; although mean sniffing duration initially rose at the same rate as in SAL-pretreated rats, it reached its plateau much earlier (at 40 min) and at much lower intensity (at approximately 31-s intervals). A 2 × 12 ANOVA highlighted the difference between SAL- and PCPA-pretreated subjects: mean durations differed significantly between pretreatment conditions, $F(1, 120) = 39.7$, $p < 0.005$, and among the 12 observation periods, $F(11, 120) = 4.6$, $p < 0.005$, with a highly significant pretreatment × period interaction, $F(11, 120) = 3.5$, $p < 0.005$. A post hoc Tukey HSD test indicated no significant differences in PCPA-pretreated rats among any observation periods; conversely, among SAL-pretreated animals, mean duration was significantly higher in each observation period from 58 to 90 min than in observation periods at the beginning and end of the testing session ($p < 0.05$).

MPH-Induced Stereotypy

The behavioral profile induced by 30 mg/kg MPH differed substantially from that induced by 5 mg/kg DEX. Table 1 lists total time engaged in gnawing behaviors across the six pretreatment/treatment conditions. Animals receiving MPH exhibited a much higher degree of gnawing behavior than those receiving saline or DEX on the test day. A 2 × 3 ANOVA confirmed this effect: main treatment effects were found to be highly significant, $F(2, 28) = 104.8$, $p < 0.005$. As with the case of DEX-induced sniffing, ANOVA also revealed a significant pretreatment effect, $F(1, 28) = 4.7$, $p < 0.05$, with PCPA attenuating gnawing behavior (as collapsed across the three treatment conditions). Pretreatment × treatment interaction just reached significance, $F(2, 28) = 3.3$, $p = 0.05$, necessitating a post hoc Tukey HSD analysis (described below). One-way ANOVA collapsed across pretreatment conditions revealed a highly significant between-groups difference, $F(2, 31) = 82.4664$, $p < 0.005$; post hoc Tukey multiple comparisons testing for the one-way ANOVA confirmed that MPH induced significantly more gnawing ($p < 0.05$) than SAL or DEX, which did not differ from each other.

Post hoc Tukey HSD analysis, which confirmed that MPH provoked significantly more gnawing than DEX or SAL in saline-pretreated animals, also indicated that PCPA pretreatment significantly decreased the gnawing effects of MPH ($p < 0.05$). PCPA failed to diminish significantly gnawing behaviors observed in either the SAL or DEX treatment conditions; however, even PCPA-pretreated animals exhibited significantly more gnawing behavior in the MPH treatment condition than 5-HT-depleted animals receiving SAL or DEX on the test day ($p < 0.05$). Recall that in the case of DEX-induced sniffing, PCPA depressed the observed behavior among DEX-treated animals to the same level as PCPA-pretreated animals in the SAL and MPH test-day conditions. It therefore appears likely that while PCPA attenuates MPH-induced

TABLE 1

TOTAL TIME IN SECONDS (± SEM) ENGAGED IN VARIOUS BEHAVIORS FOR ANIMALS IN EACH PRETREATMENT/TREATMENT CONDITION

Pretreatment/ Treatment	*n*	Lying Still	Sniffing	Gnawing
SAL/SAL	6	487.8 ± 84.3	303.0 ± 90.7	191.2 ± 80.3
PCPA/SAL	6	549.8 ± 119.9	275.5 ± 105.8	150.3 ± 55.9
SAL/DEX	6	0.0 ± 0.0	1140.8 ± 46.6	18.3 ± 4.7
PCPA/DEX	6	0.8 ± 0.8	547.3 ± 214.6	4.7 ± 2.2
SAL/MPH	5	0.0 ± 0.0	54.8 ± 12.0	1098.5 ± 85.1
PCPA/MPH	5	0.0 ± 0.0	142.6 ± 21.5	767.2 ± 112.2

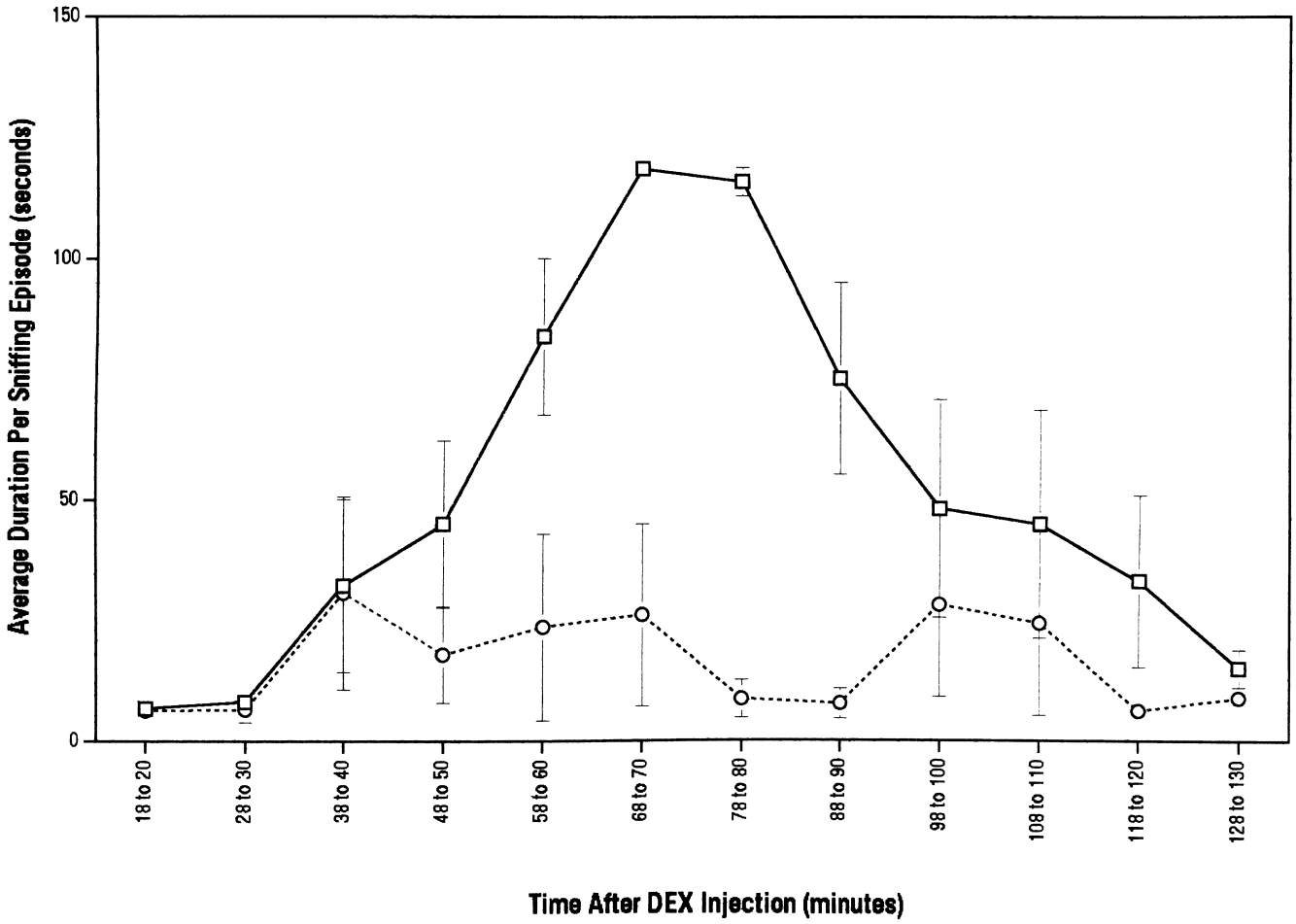

FIG. 1. Average duration per sniffing episode in DEX-treated rats, plotted as a function of time after injection. The open squares represent saline pretreated animals. The open circles represent PCPA pretreated animals.

gnawing, it does so to a lesser extent than in the case of DEX-induced sniffing.

A further comparison of the effects of saline and PCPA pretreatment on MPH-induced gnawing is found in Fig. 2, which plots mean duration per gnawing episode as a function of time for rats in the SAL/MPH and PCPA/MPH conditions. As with DEX-induced sniffing, MPH-provoked gnawing appears to constitute a multiphasic effect. PCPA constrained the overall gnawing profile, but apparently to a lesser extent than with DEX-induced sniffing: as seen in Fig. 2, average duration peaked at approximately the same time in both pretreatment conditions (around 60 min postinjection), and while the PCPA plot never truly approached the SAL plot during the period of peak intensity (reaching at most a duration of 57 s compared to 120), it certainly came closer than did the PCPA plot in Fig. 1 (31 s compared to 119). A 2×12 ANOVA indicated highly significant main effects of pretreatment, $F(1, 96) = 42.9$, $p < 0.005$, and time, $F(11, 96) = 6.8$, $p < 0.005$, on average gnawing duration; pretreatment $\times$ time interaction was also found to be significant, $F(11, 96) = 2.3$, $p < 0.05$. Post hoc epsilon tests were performed to compare the strength of the time $\times$ pretreatment interactions in DEX- and MPH-treated rats; animals in the DEX treatment condition were found to have a stronger interaction ($\epsilon = .415$) than those receiving MPH ($\epsilon = 0.347$). Moreover, unlike in the analysis of DEX-provoked sniffing duration, a post hoc Tukey HSD test comparing changes in mean gnawing duration over time did reveal a significant difference between two observation periods for PCPA-pretreated animals: mean duration from 58–60 min(57.0 s) was significantly greater ($p < 0.05$) than mean duration from 128–130 min (6.9 s). If the latter may be thought of as the baseline duration (considering both its postpeak occurrence and its proximity to the mean durations of the previous two observation periods), the Tukey HSD test indicates a significant change in the behavioral profile over time, even among PCPA-pretreated animals. This finding reinforces the notion that while PCPA significantly attenuates both DEX-provoked stereotyped sniffing and MPH-induced perseverative gnawing, its effect on the former behavior is substantially more profound than its effect on the latter.

Effect of PCPA on 5-HT

A 2×3 ANOVA was prepared to compare the effects of SAL and PCPA pretreatment on 5-HT content in the striatum, frontal cortex, and thalamus. PCPA pretreatment significantly depressed 5-HT levels across all three brian areas studied, $F(1, 57) = 5.4$, $p < 0.05$. Consistent with the observation of Dickinson and Curzon (7), who employed an identical PCPA pretreatment method and saw an 82% reduction in

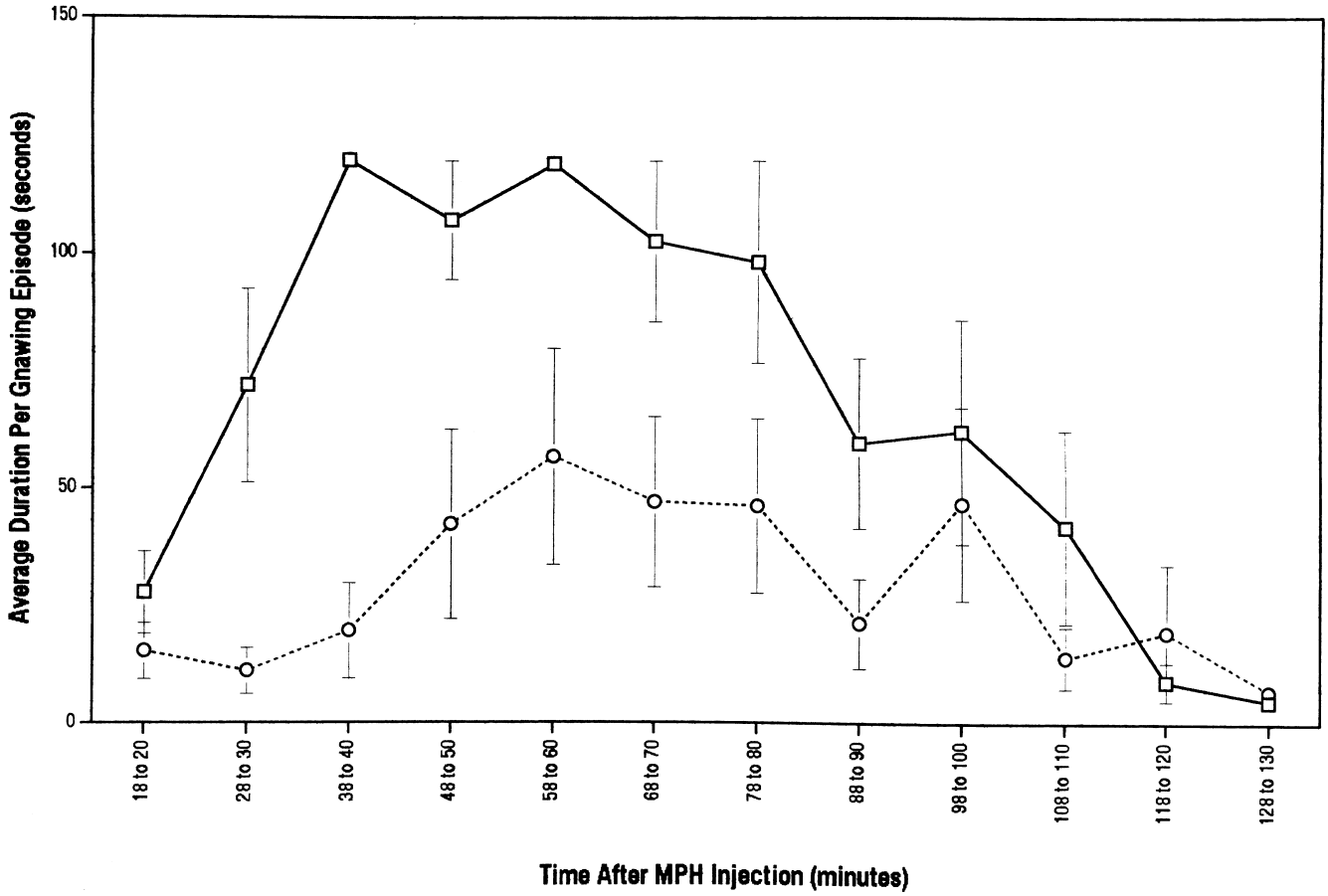

FIG. 2. Average duration per gnawing episode in MPH-treated rats, plotted as a function of time after injection. The open squares represent saline pretreated animals. The open circles represent PCPA pretreated animals.

central 5-HT, the present data indicate an overall 5-HT reduction of 89%. This depression was more pronounced in the striatum (94%) and thalamus (93%) than in the frontal cortex (82%).

A Student's *t*-test was conducted for each brain area to determine whether PCPA similarly depleted 5-HT across DEX- and MPH-treated animals. No significant differences were found in striatal, $t(3.00) = -1.00$, $p > 0.05$, cortical, $t(3.13) = -0.86$, $p > 0.05$, or thalamic, $t(3.00) = 1.00$, $p > 0.05$, 5-HT levels between DEX- and MPH-rats receiving PCPA pretreatment.

DISCUSSION

The present findings replicate and extend the observations of Pechnick et al. (25), who first reported DEX and MPH induce differential patterns of stereotypy in rats. Preliminary discussion of which of the observed behaviors may be classified as "stereotyped" will focus on those animals receiving SAL pretreatment.

The total times animals spent engaged in putative stereotyped behaviors were compared across treatment conditions (Table 1). Animals receiving 5 mg/kg DEX demonstrated significantly more sniffing over a 2-h observation period than animals receiving either saline or 30 mg/kg MPH ($p < 0.05$); rats treated with MPH exhibited less total sniffing than saline-treated rats, but this difference did not reach significance. Conversely, MPH-treated animals displayed significantly higher total gnawing than SAL- or DEX-treated rats ($p < 0.05$); rats receiving DEX gnawed less (but not significantly less) than animals receiving saline. These two findings establish a fine contrast: while DEX significantly potentiated sniffing behavior, it did so at the expense of gnawing (and perhaps other nondocumented behaviors as well); MPH, on the other hand, increased gnawing while decreasing sniffing. Both of these findings point to a stimulant-driven reduction of overall behavioral variability in favor of focused stereotypy, in accordance with the Lyon and Robbins hypothesis (20); yet, as the data clearly indicate, MPH and DEX provoked entirely different stereotypy profiles.

Recall that Pechnick et al. (25) obtained similar results, but utilizing adult rats (180–360 g). According to Mabry and Campbell (21), neurotransmitters in the rat brain do not reach their adult levels until the age of 40 days. However, the present findings, when compared to those described by Pechnick et al., indicate that 5 mg/kg DEX and 30 mg/kg MPH exert the same behavioral effects in 26 to 30-day-old rats as in adult animals. These findings imply that the same neurochemical mechanisms underlie stimulant-specific stereotypies in young and postadolescent rats. Moreover, to the extent that the relatively immature CNS of the young rat parallels that of the human child, the present findings suggest a more suitable model for the differential side effects of DEX and MPH in ADHD children than do the observations of Pechnick et al.

This experiment also broadened the scope of the initial observations reported by Pechnick et al. (22) with respect to the neurochemical basis of stimulant-induced perseverative behaviors. Pretreatment of some animals with PCPA, an agent that decreased central serotonin by 89% when administered 48 to 24 h prior to behavioral testing, fostered a determination not only of which stereotyped behaviors are mechanistically dependent on 5-HT, but of the relative level of dependence for both DEX- and MPH-induced stereotypies. As indicated in Table 1, PCPA effected a slight overall behavioral inactivation among SAL-treated controls; however, this inactivation failed to reach significance. Moreover, as an examination of Table 1 will attest, PCPA pretreatment did not significantly affect sniffing or gnawing behaviors in rats given saline on the test day (although it did have the effect of slightly decreasing the duration and total occurrence of both of these activities). Thus, by itself, PCPA pretreatment failed to alter substantially either the general activity level or the expression of specific behaviors in saline-treated animals.

Conversely, the effect of PCPA on DEX-induced sniffing was quite pronounced. As seen in Table 1, pretreatment with PCPA significantly reduced total sniffing behaviors among animals given DEX on the test day ($p < 0.05$). In fact, aside from exhibiting a decreased tendency to remain still, PCPA/DEX rats closely resembled PCPA/SAL rats in their overall behavior. Figure 1 provides additional evidence that suggests that stereotyped, DEX-induced sniffing is strongly dependent on serotonergic mechanisms: pretreatment with PCPA resulted in the highly significant constriction of the overall sniffing profile over time ($p < 0.005$), eliminating the multiphasic effect seen with animals in the SAL/DEX condition. Moreover, no significant differences in sniffing duration were seen between any two observation periods in PCPA-pretreated rats.

PCPA also attenuated MPH-induced gnawing, but not to the same extent as it affected DEX-provoked gnawing. Table 1 indicates that PCPA significantly reduced total gnawing time among MPH-treated rats ($p < 0.05$); however, unlike the case of DEX-induced sniffing, PCPA did not reduce this index to the level of PCPA/SAL control animals ($p < 0.05$). As seen in a comparison of Figs. 1 and 2, although serotonin depletion significantly reduced the variability of gnawing behavior over time ($p < 0.05$), the reduction here is not as dramatic as with DEX-induced sniffing ($p < 0.005$); comparison of epsilon values for pretreatment $\times$ time interactions among DEX-treated ($\epsilon = 0.415$) and MPH-treated ($\epsilon = 0.347$) animals confirms this effect. Furthermore, as indicated by the significant difference between peak and baseline gnawing durations among PCPA-pretreated animals in Fig. 2 ($p < 0.05$), animals in the PCPA/MPH condition still exhibited a multiphasic gnawing pattern over time (albeit a diminished one); this observation should be contrasted with the finding that serotonin depletion completely abolished the multiphasic pattern seen in SAL/DEX animals (Fig. 1). Finally, it should be noted that because no significant differences in serotonin levels between PCPA/DEX and PCPA/MPH animals were found in any brain region studied, the different behaviors exhibited by these groups cannot be attributed to differential 5-HT depletion.

These findings collectively suggest that while both *d*-amphetamine- and methylphenidate-induced stereotypies are dependent on central 5-HT, this dependence is far greater for DEX-provoked sniffing than for MPH-provoked gnawing. These results are consistent with several previous investigations that have asserted a facilitatory role for 5HT in stereotypy (9,18,31). At the same time, however, the present findings contradict several previously described investigations that have proposed that serotonergic mechanisms suppress stereotypy (2,7,19). Moreover, of particular interest is the model established by Rebec and Curtis (27) and supported by the findings of Kelley et al. (14), Dickson et al. (8), and Kuczenski et al. (17), which suggests that stimulants effect stereotypy by inhibiting serotonergic neurons downstream in the dorsal rapine nucleus (DRN) and thus disinhibiting dopaminergic neurons in the ventrolateral striatum (VLS).

Although limited by a relatively small sample size, the results of this study support the notion that the modulatory role of serotonin differs among DEX- and MPH-induced stereotypies. Although it is difficult to compare the qualitatively different behaviors provoked by DEX and MPH in laboratory rats to the divergent behavioral profiles seen among ADHD children given these same stimulants (3), these results do insinuate a mechanistic similarity: the greater dependence of DEX-induced sniffing on 5-HT suggests one possible model for the OCD-like behaviors provoked by *d*-amphetamine treatment in children with ADHD, especially given the strong evidence linking perfectionistic and ritualistic behaviors in humans to increased serotonin levels (10,13,32,33).

Although this study has attempted to clarify the neurochemical mechanisms that mediate *d*-amphetamine- and methylphenidate-induced stereotypies, further work in this area is required to develop a more complete understanding of how these stimulants influence perseverative behaviors. Such research could lay the groundwork for the evolution of new drug therapies designed to correct the debilitating symptoms of ADHD with fewer deleterious side effects.

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