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Locomotor Stereotypy is Produced by Methylphenidate and Amfonelic Acid and Reduced by Haloperidol but Not Clozapine or Thioridazine

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MUELLER, K. *Locomotor stereotypy is produced by methylphenidate and amfonelic acid and reduced by haloperidol but not clozapine or thioridazine.* PHARMACOL BIOCHEM BEHAV **45**(1) 71–76, 1993.—In addition to its well-known behavioral effects in rats, amphetamine also produces patterned locomotion (referred to below as locomotor stereotypy) in an open field. Locomotor stereotypy may be mediated by different mechanisms than those mediating the better-known behavioral effects of amphetamine. To determine whether the ability to produce locomotor stereotypy is an exclusive property of amphetamine or is a property of many amphetamine-like stimulants, several doses of methylphenidate and amfonelic acid were tested. The ability of both atypical and typical neuroleptics to block amphetamine-induced locomotor stereotypy was also tested. Both amfonelic acid and methylphenidate produced some degree of locomotor stereotypy. In addition, amphetamine-induced locomotor stereotypy was reduced by haloperidol but not by clozapine or thioridazine. These data suggest that locomotor stereotypy is more closely related to focused stereotypy than to hyperlocomotion.

Amfonelic acid Methylphenidate Clozapine Haloperidol Thioridazine Amphetamine Locomotor stereotypy

AMPHETAMINE-induced locomotor stereotypy refers to the patterned locomotion exhibited by amphetamine-treated rats. Although other amphetamine-induced behaviors have been well characterized (both behaviorally and neurochemically), much less is known about locomotor stereotypy. A critical role of striatal dopamine would seem logical; however, some data suggest that amphetamine-induced hyperlocomotion, locomotor stereotypy, and focused stereotypy (absence of locomotion and sniffing/licking of a restricted portion of the environment) are mediated by distinct neurochemical mechanisms. In the research described below, stimulants that are neurochemically dissimilar from but behaviorally similar to amphetamine were tested for their ability to produce locomotor stereotypy. In addition, dopamine antagonists with different profiles of action were tested for their ability to block amphetamine-induced locomotor stereotypy.

Amphetamine produces reliable behavioral changes in rats. Lower doses increase locomotion and sniffing by increasing release of dopamine in mesolimbic areas. Higher doses produce focused stereotypy by increasing release of dopamine by the nigrostriatal system (5,6,10). Locomotor stereotypy appears after a wide range of doses of amphetamine but fails to appear after doses of caffeine that produce equal or greater hyperlocomotion (14). Because caffeine and amphetamine produce hyperlocomotion by different mechanisms (9), locomotor stereotypy is not an artifact of hyperlocomotion per se. Because locomotor stereotypy combines aspects of both hyperlocomotion and stereotypy, the mechanisms underlying these behaviors may well be similar. Likewise, because most amphetamine-induced behaviors are mediated by increased activity in either mesolimbic or nigrostriatal dopamine systems, these same systems are likely candidates for the mediation of locomotor stereotypy.

However, some data are inconsistent with this hypothesis. Apomorphine acts on dopamine receptors and produces focused stereotypy but fails to produce locomotor stereotypy in an open field (16) [although it does produce repetitive locomotion in a much smaller testing arena (7)]. Scopolamine, a cholinergic antagonist, produces both hyperlocomotion and locomotor stereotypy but fails to produce focused stereotypy (16). Thus, focused stereotypy and locomotor stereotypy may be mediated by different neurochemical mechanisms.

One way to begin to address the problem of the neurochemical mechanisms underlying locomotor stereotypy is to

screen various drugs for the ability to produce locomotor stereotypy and to block amphetamine-induced locomotor stereotypy. Because each drug has a slightly different neurochemical profile, clues to the mechanism of locomotor stereotypy would be provided.

Both amfonelic acid and methylphenidate produce amphetamine-like behaviors; that is, both drugs produce hyperlocomotion and focused stereotypy. In fact, amfonelic acid and amphetamine are so similar behaviorally that amfonelic acid substitutes for the amphetamine cue in drug-discrimination tasks (1). Despite the similarities in the behavioral profiles, there are consistently reported differences between the pharmacological profiles of these stimulants.

For example, the behavioral effects of amphetamine are more sensitive to α-methylparatyrosine than to reserpine but the behavioral effects of methylphenidate and amfonelic acid are more sensitive to reserpine than to α-methylparatyrosine (4). These three agents also differ in their effect on accumulation of striatal DOPA: Amphetamine increases the accumulation of striatal DOPA, methylphenidate reduces the accumulation of striatal DOPA, and amfonelic acid fails to affect the accumulation of striatal DOPA (11,12). Thus, in the first series of experiments described below, a dose–response study was conducted to determine whether methylphenidate and amfonelic would produce locomotor stereotypy.

Dopamine antagonists also differ in their ability to normalize amphetamine-induced behaviors. Haloperidol is a dopamine D_2 antagonist that is also classified as a typical antipsychotic. Haloperidol blocks both amphetamine-induced hyperlocomotion and amphetamine-induced focused stereotypy. However, the dopamine antagonists clozapine and thioridazine (which are also called atypical antipsychotics) fail to block amphetamine-induced focused stereotypy (3,20). If these atypical antipsychotics block locomotor stereotypy, strong evidence would be provided that locomotor stereotypy and focused stereotypy are mediated by different mechanisms. Thus, in the second series of experiments described below several doses of clozapine, haloperidol, and thioridazine were compared for the ability to block amphetamine-induced locomotor stereotypy.

METHOD

Animals

Male albino rats (350–470 g) were bred from Wistar stock at the departmental vivaria. Rats were housed individually in standard wire cages with food and water available ad lib. A 24 L : 24 D cycle was maintained; testing was always conducted 2 h prior to lights out.

Open Field and Data Reduction

All testing was conducted in an open field 112 × 112 × 30 cm. The floor of the open field was divided into five equal areas: a circle in the center and four surrounding areas. The rat's path through the open field was traced onto a schematic of the open field. The rat's path through the open field was then divided into a series of trips [for explanation, see (14,15)]. The index of locomotor stereotypy, $\hat{\gamma}$, is calculated by dividing the total number of (sequentially) repeated trips by the total number of trips exhibited. Thus, $\hat{\gamma}$ quantifies the probability that the rat will repeat the trip that it has just exhibited; higher values indicate greater locomotor stereotypy. Note that data on locomotor stereotypy cannot be collected in the absence of locomotion; a $\hat{\gamma}$ score of 0 indicates the absence of sequentially repeated trips, not the absence of locomotion. For example, a rat that fails to locomote at all during a given observation interval can be assigned a score of "0" for lines crossed, but the $\hat{\gamma}$ score is essentially "missing." Recall that a $\hat{\gamma}$ score of 0 means absence of repeated trips, not absence of locomotion. Monte Carlo simulations of the sampling distribution of $\hat{\gamma}$ have indicated that rats that exhibit three or less trips during a given observation period should be assigned "missing data."

Design

The design of both series of experiments is the same. The dependent variables (lines crossed and $\hat{\gamma}$) are reported in 10-min intervals. Differences across time periods and between groups were evaluated with a two-factor mixed analysis of variance (ANOVA) (where dose is the between-groups variable and time after injection is the repeated measure). For lines crossed, BMDP2V (8) was used. Because of the problem of missing data, BMDP5V was used for the $\hat{\gamma}$ data (18). The latter program used a Wald-type significance test (which in turn is based upon a χ^2 distribution) to assess differences between groups and time periods. It enables use of $\hat{\gamma}$ data from a rat that is missing one or two $\hat{\gamma}$ values because of failure to locomote during particular time periods.

Testing

Each rat was briefly handled once a day for 5 days prior to testing. Rats were placed in the open field for a 40-min habituation period 48 h prior to testing and again 24 h prior to testing. On the day of testing, rats were placed in the center area of the open field immediately after injection with the stimulant of interest. Rats were videotaped for varying periods of time, depending upon the dose and half-life of the drug of interest. Each rat was tested only once. Videotapes were scored by trained assistants; random tapes were scored by an independent observer to evaluate interrater reliability. Correlations coefficients were extremely high (from 0.97–0.99).

Experiments

The first series of experiments tested the ability of several doses of methylphenidate (vehicle n = 7, 1 mg/kg n = 8, 2 mg/kg n = 10, 3 mg/kg n = 8, 4 mg/kg n = 9, 6 mg/kg n = 9) and amfonelic acid (vehicle n = 8, 0.5 mg/kg n = 7, 0.75 mg/kg n = 8, 1.0 mg/kg n = 8, 1.25 mg/kg n = 7, 1.5 mg/kg n = 8) to produce locomotor stereotypy. The data from each drug were analyzed separately; that is, the dose–response study of methylphenidate and amfonelic acid were treated as separate experiments. Each drug was injected SC. Methylphenidate (Sigma Chemical Co., St. Louis, MO) was dissolved in normal saline. Amfonelic acid (Sigma) was dissolved in sodium hydroxide; the pH was then adjusted with dilute HCl.

The second series of experiments tested the ability of several doses of dopamine antagonists to block amphetamine-induced locomotor stereotypy. Data from each dopamine antagonist were analyzed separately. Several doses of haloperidol (vehicle, 0.0125 mg/kg, 0.0325 mg/kg, 0.040 mg/kg), thioridazine (vehicle, 2.5 mg/kg, 10 mg/kg), and clozapine (vehicle, 1 mg/kg, 2 mg/kg) (n = 12 for each dose of each drug) were injected 30 min prior to injection with 2 mg/kg amphetamine sulfate (Sigma). Clozapine (graciously provided by Sandoz, East Hanover, NJ) and haloperidol (Sigma) were dissolved in a small amount of lactic acid and diluted with saline.

RESULTS

Methylphenidate and Amfonelic Acid

As expected, both methylphenidate and amfonelic acid increased lines-crossed as compared to vehicle (see Fig. 1). Results of the statistical analyses are as follows: amfonelic acid, main effect of dose not significant; main effect of time after injection, $F(11, 297) = 10.04$, $p < 0.01$; dose × time interaction, $F(5, 45) = 4.40$, $p < 0.01$; methylphenidate, main effect of time after injection, $F(5, 222) = 4.22$, $p < 0.01$; dose × time interaction, $F(25, 225) = 2.30$, $p < 0.01$.

Both methylphenidate and amfonelic acid increased $\hat{\gamma}$ scores over time, $\chi^2(8) = 58.03$, $p < 0.01$; $\chi^2(8) = 31.34$, $p < 0.01$, for the main effect of time for amfonelic acid and methylphenidate, respectively. The main effect of dose was significant, $\chi^2(3) = 16.23$, $p < 0.001$, only for amfonelic acid. The dose × time interactions were not significant in either case. Note that vehicle-treated rats exhibited insufficient locomotion for calculation of $\hat{\gamma}$ scores at all but the first 10-min interval after injection (0.170 ± 0.050 and 0.245 ± 0.060 are the mean and SE for the methylphenidate and amfonelic acid vehicle groups, respectively); therefore, these data are not shown.

Haloperidol, Thioridazine, and Clozapine

As expected, amphetamine increased lines crossed over time (see Fig. 2). Even low doses of haloperidol dramatically reduced the effect of amphetamine on lines crossed, $F(15, 220) = 6.03$, $p < 0.01$, for the dose × time interaction. In general, the higher the dose of haloperidol, the greater the reduction in lines crossed.

On the other hand, thioridazine failed to significantly affect amphetamine-induced locomotion; that is, the main effect of dose and the dose × time interaction are not significant). The higher dose of clozapine (2 mg/kg) dramatically reduced the effect of amphetamine on lines crossed; however, the lower dose of clozapine (1 mg/kg) actually enhanced the effects of amphetamine on lines crossed, $F(10, 165) = 2.74$, $p < 0.01$, for the dose × time interaction. Haloperidol re-

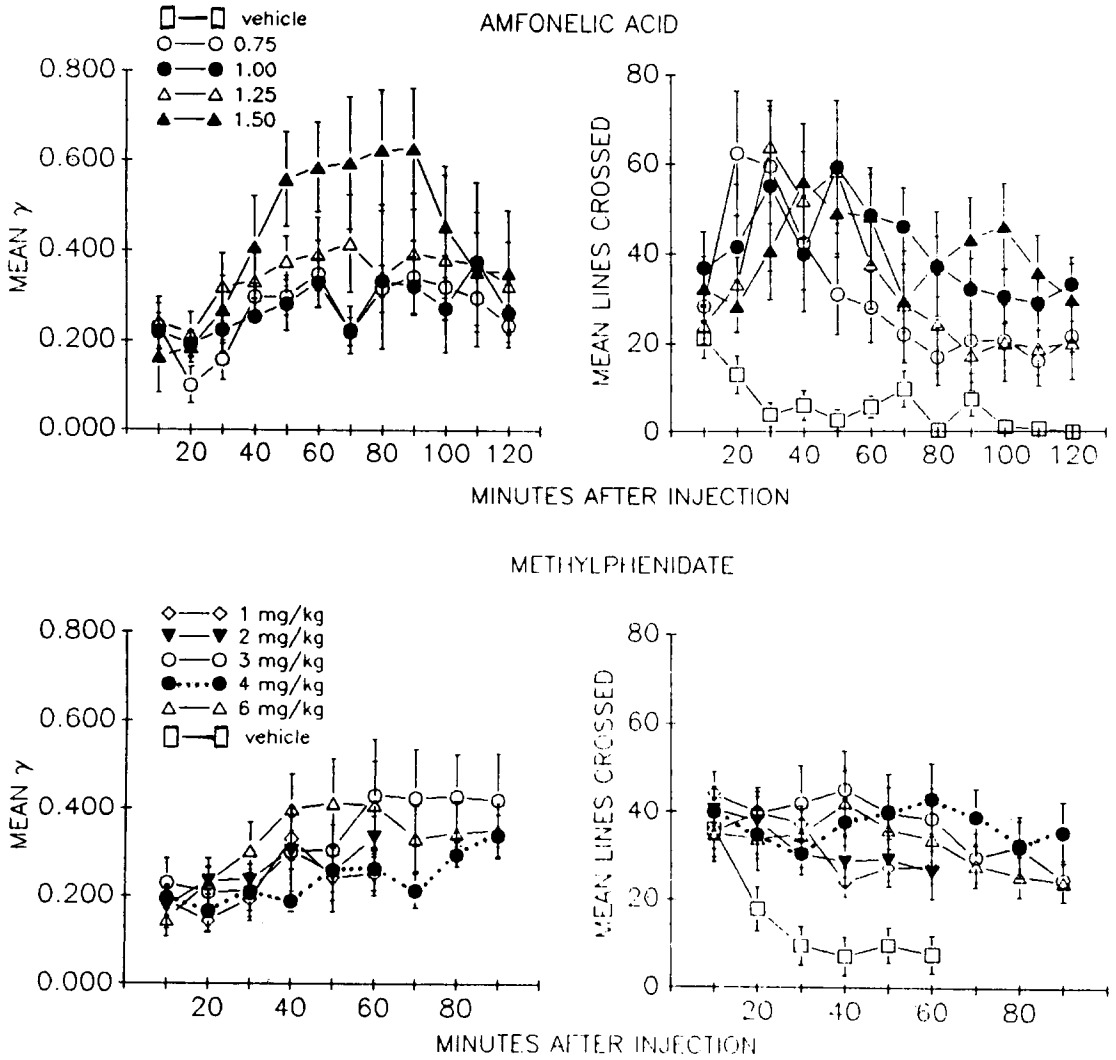

FIG. 1. Effects of methylphenidate (right) and amfonelic acid (left) on lines crossed (top) and locomotor stereotypy (bottom). Note that rats injected with lower doses of drugs are observed for shorter periods of time.

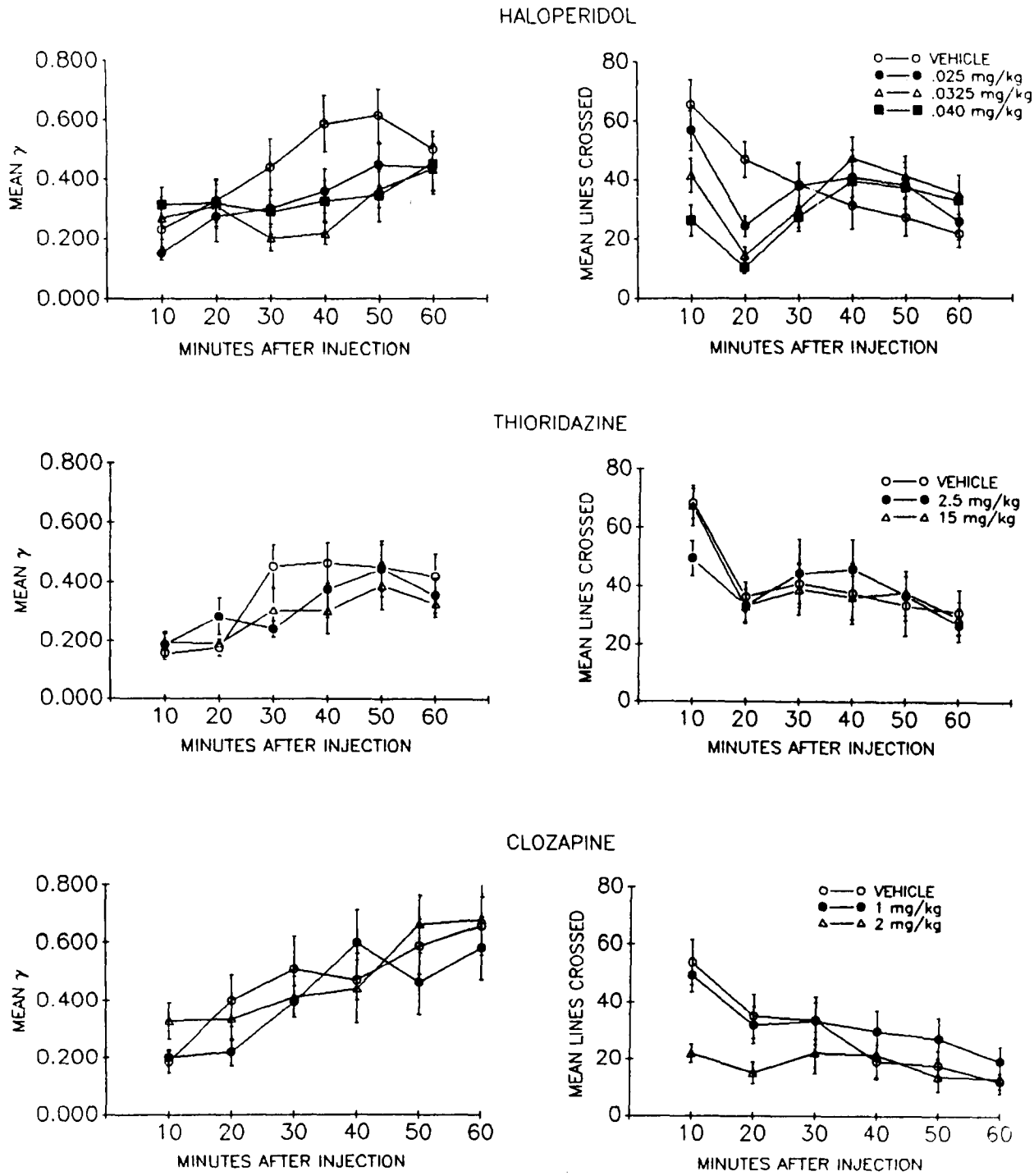

FIG. 2. Effects of haloperidol (top), thioridazine (middle), and clozapine (bottom) on amphetamine-induced locomotor stereotypy and hyperlocomotion.

duced $\hat{\gamma}$ scores, $\chi^2(15) = 25.06$, $p < 0.05$, for the dose × time interaction, but thioridazine and clozapine did not.

DISCUSSION

Of the antipsychotics tested, only haloperidol blocked amphetamine-induced locomotor stereotypy. The stimulants amfonelic acid and methylphenidate both produced at least some degree of locomotor stereotypy. Further, both produced locomotor stereotypy that was similar to amphetamine-induced locomotor stereotypy; that is, in the majority of cases locomotor stereotypy was the result of repetitive trips from one end of the open field to the other.

Although the higher dose of amfonelic acid clearly produced locomotor stereotypy, the methylphenidate data are more difficult to interpret. Because vehicle-treated rats fail to exhibit sufficient locomotion for calculation of $\hat{\gamma}$ during most observation periods, a comparison of $\hat{\gamma}$ scores between drug- and vehicle-treated rats is impossible. The observation that methylphenidate increased $\hat{\gamma}$ scores over time could be evidence that methylphenidate produced locomotor stereotypy. On the other hand, a simple main effect of time after injection could also mean that $\hat{\gamma}$ scores were suppressed initially and gradually returned to control levels. This interpretation is not likely in the present case, however. Notice that initial $\hat{\gamma}$ scores (10 min after injection) are similar for all doses of methylphenidate, amfonelic acid, and vehicle + amphetamine and for vehicle-treated rats. Therefore, the conclusion that methylphenidate indeed produces locomotor stereotypy seems warranted.

However, amphetamine seems to produce more obvious locomotor stereotypy and more consistent locomotor stereotypy across rats than either methylphenidate or amfonelic acid [compare Figs. 1 and 2 and see (15)] although, admittedly, the experiments were not designed to test such a hypothesis. Increasing the dose of methylphenidate or amfonelic acid would be counterproductive because locomotion is incompatible with focused stereotypy. Thus, although ability to produce locomotor stereotypy is apparently a property of a wide range of amphetamine-like stimulants, some property of amphetamine apparently makes it more efficient in this regard.

As expected, haloperidol reduced both amphetamine-induced hyperlocomotion and locomotor stereotypy. However, the dose-response curves for $\hat{\gamma}$ and lines crossed were different. In general, increasing the dose of haloperidol increased its effectiveness at blocking amphetamine-induced changes in lines crossed. However, the three doses of haloperidol were similar to each other in their ability to reduce $\hat{\gamma}$ scores. Note that the doses of haloperidol used here are much lower than those commonly used.

Unexpectedly, clozapine and thioridazine failed to block the amphetamine-induced increase in $\hat{\gamma}$ scores even though clozapine was efficient at blocking hyperlocomotion. In fact, higher doses of clozapine could not be used because of the severe depression of locomotion. The doses of thioridazine used here are well within the range of doses used in other studies; thioridazine has not consistently reduced amphetamine-induced lines crossed in other studies (3,20).

The failure of clozapine and thioridazine to reduce $\hat{\gamma}$ scores is important because these drugs either fail to suppress amphetamine-induced focused stereotypy or actually enhance amphetamine-induced focused stereotypy (17). Thus, the inability of clozapine and thioridazine to suppress amphetamine-induced locomotor stereotypy is consistent with the hypothesis that amphetamine-induced locomotor stereotypy and amphetamine-induced focused stereotypy are mediated by similar neurochemical mechanisms. The different profiles of action of haloperidol, clozapine, and thioridazine may provide clues to the mechanisms underlying locomotor stereotypy.

One of the popular explanations for differences between the typical and atypical neuroleptics is that the typical neuroleptics act in both the nucleus accumbens and caudate while the atypical neuroleptics act only in the nucleus accumbens [cf. (2)]. This hypothesis suggests that amphetamine-induced locomotor stereotypy is mediated by the caudate, not by the nucleus accumbens. On the other hand, the hypothesis about selective action of atypical neuroleptics in the nucleus accumbens has become controversial.

Differential activity at subclasses of dopamine receptors is now the most popular explanation for the different actions of dopamine blockers (13). Interestingly, haloperidol has far more selectivity for D_2 receptors than does thioridazine or clozapine (19). These data suggest the hypothesis that amphetamine-induced locomotor stereotypy might be a D_2-mediated phenomenon.

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