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Day and Night Locomotor Activity Effects During Administration of (+)-Amphetamine

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MARTIN-IVERSON, M. T. AND S. D. IVERSEN. *Day and night locomotor activity effects during administration of (+)-amphetamine.* PHARMACOL BIOCHEM BEHAV **34**(3) 465–471, 1989.—Rats were given continuous subcutaneous amphetamine infusions (0, 2, 6, 10 and 20 mg/kg/day) via osmotic minipumps. The effects of these treatments on the locomotor activity of rats were determined over both light and dark phases of a 12-hr light/dark cycle for 336 consecutive hours. It was observed that tolerance to the locomotor stimulant actions of (+)-amphetamine is both dose- and light/dark cycle-dependent. Locomotor stimulation induced by the two highest doses remained high during both day and night throughout the period of treatment, except for the first few days and nights with the highest dose. Tolerance developed only to the effects of the two lower doses, and only during the day. Effects of the low doses on locomotor activity and on circadian patterns of locomotor activity are roughly similar to those previously observed with continuous administration of a selective dopamine D2 agonist. This *behavioral* similarity suggests that dopamine released by continuous administration of low doses of (+)-amphetamine may be producing its effects via selective actions on DA D2 receptors in vivo.

Rat (+)-Amphetamine Tolerance Circadian rhythms Locomotor activity Sensitization Dopamine D1/D2 receptors

IT has been recognized since at least the 1950's (2) that chronic abusers of psychomotor stimulants such as amphetamine frequently develop psychotic symptoms similar to those of paranoid schizophrenia. Stimulants exacerbate psychotic symptoms in schizophrenics, as well as inducing a psychosis in some stimulant abusers (1, 16, 19, 33). In addition, patients with Parkinson's disease may develop psychotic reactions, often with paranoid symptoms, to drug therapy with L-DOPA, a dopamine precursor (1), or with direct dopamine agonists such as bromocriptine (15,18). Animal models of stimulant psychosis are based on the rationale that chronic or subchronic treatment of animals with stimulants may produce behavioral or neurochemical effects that are homologous to the effects of these drugs in humans. Thus, investigating the effects of subchronic stimulant administration to animals may shed light on the neurological substrates of stimulant-induced psychosis.

Three major stimulant treatment regimens have been employed in such animal models. One regimen consists of intermittent injections of moderate to low doses of stimulants (usually (+)-amphetamine), once or twice daily (31). This treatment regimen produces a gradual augmentation of the motor stimulant effects of amphetamine. Little consensus has been reached as to the effects of this treatment on dopamine (DA) receptors. Unlike other treatment regimens, intermittent injections of relatively low doses of amphetamine do not produce degeneration of DA-releasing terminals. On the other hand, amphetamine-induced release of DA is increased [see (31) for a recent thorough review of the literature on the neurochemical effects of intermittent injections of low doses of amphetamine]. A second regimen also uses intermittent injections, usually with high doses or with progressively higher doses of methamphetamine, sometimes more than twice daily (32). This regimen is apparently neurotoxic to DA-releasing terminals (20,30) and results in tolerance to some motor stimulant effects (29).

The third treatment regimen consists of continuous infusions of low to moderate doses of (+)-amphetamine with subcutaneous implants of silicone pellets or Alzet osmotic minipumps (5,7). This procedure may also be neurotoxic to striatal DA terminals (7,28), although significant reductions in DA content of the striatum are not always found (5). Tolerance develops to the motor stimulant effects of (+)-amphetamine when given continuously (5, 8, 27). Some investigators have reported observations of a behavioral syndrome emerging after several days of continuous treatments of rats or monkeys with (+)-amphetamine (9). This syndrome is similar to behaviors induced with hallucinogens (9), and has been suggested to be related to the psychotomimetic effects of amphetamine. It is not presently clear if any one treatment regimen provides a better animal model of stimulant

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psychosis than the others.

Continuous administration of (+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a direct DA agonist selective for the D2 receptor subtype, leads to the development of daytime tolerance and nocturnal sensitization to its locomotor stimulant effects (23–25), while intermittent injections produce daytime behavioural sensitization (25). Thus, continuous infusions of this DA agonist via osmotic minipumps appear to produce behavioral characteristics of both the intermittent and continuous amphetamine treatment regimens, depending upon the phase of the light-dark cycle. It is relevant to animal models of stimulant psychosis to determine whether or not continuous amphetamine treatment also produces both light-cycle-dependent sensitization and tolerance.

In the present experiments, a range of doses of (+)-amphetamine (0, 2, 6, 10 and 20 mg/kg/day) was administered to rats over 14 (or 15 including the day of implants) days using subcutaneous implants of Alzet osmotic minipumps. These doses were chosen on the basis of intermittent injection studies by the authors, from a literature review, and from previous experience comparing the effects of intermittent injections to continuous infusions with other psychomotor stimulants. The doses chosen included those estimated to be optimum for locomotor stimulation or focussed oral stereotypies. Locomotor activity was monitored by counting photobeam interruptions throughout the period of drug treatments. The effects of different doses of (+)-amphetamine on locomotor activity were assessed during both phases of the 12-hr light/dark cycle. In addition, the effects of "arousing stimuli" associated with the normal maintenance of the animals' living conditions were assessed, as this was previously shown to reverse daytime tolerance to the effects of PHNO (23–25). Body weights of the rats were determined before and after drug treatments to determine the chronic effects of (+)-amphetamine on body weight gain (or loss).

METHOD

Animals, Materials and Basic Method

Male Sprague-Dawley rats (Bantin and Kingman, UK), n = 6 for each group except vehicle (n = 12), weighing an average of 386 g (at the initiation of drug treatments) were individually housed in wire grid cages each equipped with two sets of infrared photocells which allowed continuous monitoring of locomotor activity. The cage dimensions were 24 (w) × 16 (h) × 26 (l) cm, with two photocells placed 3 cm from the floor, and spaced 13 cm apart, equidistant from either end wall. The positioning of the photocell beams was such that it was unlikely that they would be interrupted by small movements associated with stereotypies, although this possibility could not be entirely ruled out. The effects of a variety of types and doses of stimulants on photobeam interruptions in these cages are very similar to those observed in similar cages equipped with photobeam assemblies that do not respond to frequent repeated interruptions (personal observations). The rats lived in these cages (under a light/dark cycle with the light on between 0900 and 2100 hr) for at least 10 days prior to any drug treatments. Drug treatments were continued for 15 days (including the day of implants), with continuous recording of photobeam interruptions (activity counts). On Mondays, Wednesdays and Fridays beginning a few min past 0900, the rats were given fresh water and food, and the litter underneath the cages was changed. This relatively noisy procedure lasted about 20 min and constitutes the "arousal stimuli." Food and water and the state of the animals were checked on a daily basis. Activity counts were recorded in blocks of 1 hr by a Hewlett Packard microcomputer. Average activity counts were calculated over blocks of 12 hr (nocturnal activity), 10 hr (diurnal activity) and 2 hr (time during and 100 min following the arousal stimuli, or the equivalent times on days without arousal stimuli).

Drug Administration Procedure

After 10 days of habituation to the housing conditions, the rats were implanted subcutaneously with Alzet osmotic minipumps (model 2ML2) at about 1500–1600 hr (Day 0 of drug treatments). The rats were anaesthetized with ether, a small incision was made in the dorsal midscapular region of the skin, a pocket formed under the skin and the pump or pumps inserted. The incision was closed with suture clips. Six rats were given one pump containing vehicle (polyethylene glycol), and another six rats were given two pumps containing vehicle. These 12 rats together constituted the vehicle-treated group. Eighteen rats were given pumps containing (+)-amphetamine sulfate (Smith Kline & French) dissolved in polyethylene glycol to give delivery rates of 2, 6 or 10 mg/kg/day (6 per group), based on the average weights of the animals. The remaining 6 rats were given two pumps, each delivering 10 mg/kg/day for a total of 20 mg/kg/day. All pumps were incubated in a physiological saline solution at 37°C for 4 hr prior to implantation.

Statistics

Daily and nightly activity counts were analyzed with MANOVA from the SPSS PC statistical package. The hypothesis that there was no covariance across levels of the repeated measure factor (a critical assumption underlying analysis of variance with repeated measures) was assessed with Mauchley's sphericity test (27) and was found to be violated in locomotor measures taken during both day and night. It should be noted that it is usually the case in both drug and learning research that this assumption of analysis of variance is violated in designs involving repeated measures, and this has been noted in all standard text books on statistics above the introductory level that we have perused. There are two basic strategies in dealing with this violation of the ANOVA, one that entails adjusting the degrees of freedom, and one that utilizes a multivariate approach that does not require the assumption in the first place. Treatises comparing both methods have been previously published (including one in a psychopharmacology journal): two good, easily understood ones are by McCall and Appelbaum (21) and Vetaliano (35). Both strategies have been incorporated in most good computer statistical packages, such as SPSS and BMDP. In this study, we have adopted the multivariate approach. Thus, all terms involving repeated measures were compared to results from a variety of multivariate tests (Palais trace, Hotellings T and Wilks Lambda) which do not assume a lack of covariance across repeated measures and homogeneous variances. In cases in which there was not agreement between the multivariate tests and the MANOVA results or among the multivariate tests, the raw data were subjected to reciprocal transformations, as the standard deviations were found to be relatively proportional to the square of the treatment means (10), and the MANOVA was performed on the transformed data. Individual comparisons were made with one-tailed Dunnett's tests.

The data were also analyzed by standard circadian rhythm techniques. This was done because of certain advantages of this method of analysis. Rhythm analysis allows the determination of shifts in periods, phases, amplitude and mesors of rhythms. Most of these properties of rhythms cannot be readily discerned without rhythm analysis. Since there has been evidence presented that stimulants can alter some of these characteristics of circadian rhythms (14), it is of importance to determine whether alterations in responses across the day and night periods may reflect shifts in the period or phase of the basic light cycle-driven 24-hour rhythm.

Analysis of circadian rhythms in activity counts was accomplished by Fast Fourier Transformation (Statgraphics) of the raw hourly activity counts of each animal to determine the amplitude and acrophase. Mesors were determined by taking the arithmetic average of each rat's data, beginning from the first hr of the first night, and including all data points for 336 consecutive hr. The amplitudes, acrophases and mesors were subjected to ANOVA (Statgraphics). The plots of the rhythms were determined by Halberg's cosinor method (12), using the group means of the amplitudes, phases and mesors. In brief, the acrophase is the time of the peak of the rhythm; changes in the acrophase indicate shifts in the phase of the circadian rhythm. The amplitude is the distance of the peak (or the trough) of the rhythm from the mesor, and therefore reflects the magnitude of the rhythm. The mesor (in the present case) is the mean level of activity around which the rhythm fluctuates. The period is the actual frequency of the rhythm. The Fast Fourier Transform procedure allows the determination of a wide number of possible rhythms. In this study, only the 24-hour rhythm was selected for analysis by the cosinor method. The cosinor approach allows the determination of the basic characteristics of a cyclic process, abstracting the cyclic or rhythmic process from the background variability associated with other rhythms or other noncyclic factors.

RESULTS

Effects of (+)-Amphetamine on Body Weights

The weights of all animals were determined on the days of the pump implants, and then again on the day after the last day of treatment. The overall mean of body weights before treatment was 386 ± 3.8 (SEM) g. There was no significant difference among the different groups before treatment. After treatment, body weight means and SEM's in g were altered as follows in percentage change from pretreatment values: vehicle $= 118 \pm 3.1$; 2 mg/kg/day $= 111.3 \pm 3.4$; 6 mg/kg/day $= 116.0 \pm 1.8$; 10 mg/kg/day $= 99.5 \pm 1.75$; 20 mg/kg/day $= 97.5 \pm 1.75$. Analysis of variance revealed a significant effect of dose, $F(4,11) = 11.9$, $p<0.001$. Individual comparisons revealed that only the two highest doses produced less body weight gain than the vehicle group.

Effects of (+)-Amphetamine on Daytime Activity

Figure 1 depicts the effects of the various doses of (+)-amphetamine on the average hourly activity counts during the day. There was a significant Dose × Day effect with $F(56,434) = 2.29$, $p<0.001$. Multivariate analysis verified that this significance level is appropriate. All doses of (+)-amphetamine increased activity counts on the first day of pump implants (Day 0). No further significant differences occurred between the groups receiving the lowest two doses and the vehicle-treated group from Day 1 to the last day of treatment. Activity counts in the group receiving the highest dose (20 mg/kg/day) also decreased on the second day of treatment (Day 1), but increased on subsequent days such that Days 5–14 were significantly above the vehicle group. The group receiving the 10 mg/kg/day dose exhibited a biphasic activity pattern, without significant differences from the vehicle group on Days 6 or 7, and again on 13 or 14.

Effects of (+)-Amphetamine on Nocturnal Activity

Average hourly nocturnal activity in groups of rats receiving (+)-amphetamine can be observed in Fig. 2. Since agreement was not reached between the results of analysis of variance and all of the multivariate tests, the analysis was performed on the transformed data, as described in the Statistics section. This analysis

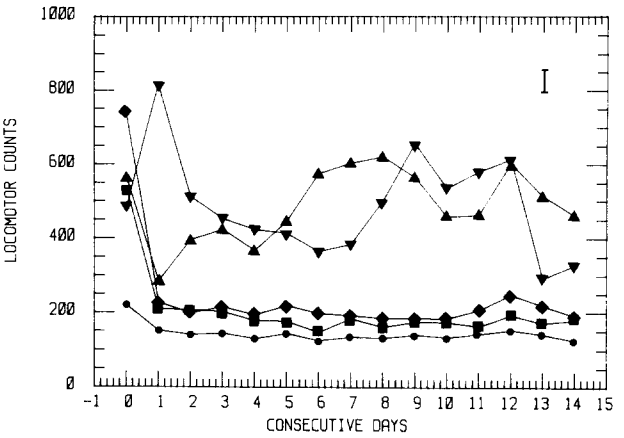

FIG. 1. Effects of a series of doses of (+)-amphetamine on rats' average hourly photobeam interruptions during each of 15 successive days (light periods; Day 0 is the day of osmotic pump implants). Rapid and sustained tolerance was observed only in the groups receiving one of the two lower doses (2 or 6 mg/kg/day). (+)-Amphetamine doses (mg/kg/day): vehicle (circles), 2.0 (squares), 6.0 (diamonds), 10.0 (inverted triangles) and 20.0 (triangles). The bar in the upper right depicts the standard error of all the means.

revealed a significant Dose × Night interaction effect with $F(52,403) = 2.29$, $p<0.001$, which was verified by all of the multivariate tests, the most conservative of which indicated $p<0.05$. In this case, the increase in activity counts produced by the two lower doses relative to the vehicle group remains relatively constant over all nights [2 mg/kg/day: $F(4,31) = 23.4$, $p<0.001$; 6 mg/kg/day: $F(4,31) = 20.8$, $p<0.01$]. The activity counts exhibited by the group receiving 20 mg/kg/day dropped to vehicle levels on Night 2 and 3, but progressively increased significantly over activity levels of the vehicle group from that point until Night 8. Activity counts of the group receiving 10 mg/kg/day (+)-amphetamine remained consistently above the vehicle group. This group

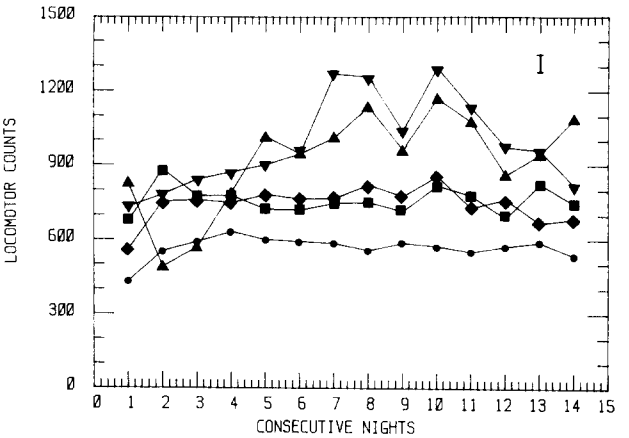

FIG. 2. Effects of a series of doses of (+)-amphetamine on rats' average hourly photobeam interruptions during each of 14 successive nights. Tolerance was not observed in any of the groups, except that receiving the highest dose on Days 2 and 3. (+)-Amphetamine doses (mg/kg/day): vehicle (circles), 2.0 (squares), 6.0 (diamonds), 10.0 (inverted triangles) and 20.0 (triangles). The bar in the upper right depicts the standard error of all the means.

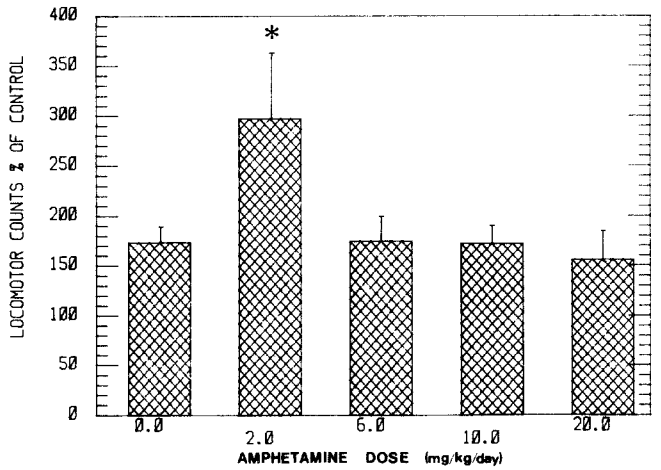

FIG. 3. Effects of a series of doses (mg/kg/day) of (+)-amphetamine on rats' percent increase in photobeam interruptions (+SEM) induced by presentation of arousal stimuli (see text for details). 'Control' refers to photobeam interruptions by the same animals at equivalent times on days without arousal stimuli. Only the lowest dose of amphetamine significantly (*$p<0.05$) increased arousal stimuli-induced photobeam interruptions over that observed in the vehicle group.

exhibited a progressive increase until Night 8, after which point there was an increase in night-to-night variability with no consistent trends apparent.

Effects of (+)-Amphetamine on Activity Induced by Arousal Stimuli

The arousal stimuli increased activity in all groups (Fig. 3). There was a significant effect of dose on the percentage increase over activity levels at the same time on days without arousal stimuli, $F(4,31)=5.20$, $p<0.005$. Only the increase in activity induced by the lowest dose was significantly greater than that produced by arousal stimuli in the vehicle group. While the data shown were collapsed over the 15 days of drug treatments, inspection of each day with arousal and the previous and subsequent days without arousal indicates the same pattern of results as shown by the collapsed data.

Effects of (+)-Amphetamine on Circadian Rhythms in Activity Counts

Figure 4 depicts double plots of the cosinor functions for the 24-hr rhythms of activity counts during continuous infusions with various doses of (+)-amphetamine. Analysis of variance revealed a significant effect of dose on the mesor [Mesor means ± SEM: 370 ± 15 (vehicle), 484 ± 19 (2 mg/kg/day), 507 ± 41 (6 mg/kg/day), 795 ± 112 (10 mg/kg/day), 760 ± 45 (20 mg/kg/day); $F(4,31)=15.3$, $p<0.0001$]. Individual comparisons indicated that both of the highest two doses significantly increased the mesor over that exhibited by the vehicle group, while the increases in mesor produced by the lowest two doses were not significant. Effects of amphetamine on the amplitude were close to but not at the 0.05 level of significance [Amplitude means ± SEM: 242 ± 18 (vehicle), 317 ± 22 (2 mg/kg/day), 341 ± 43 (6 mg/kg/day), 302 ± 29 (10 mg/kg/day), 322 ± 39 (20 mg/kg/day); $F(4,31)=2.27$, $p<0.1$]. Individual comparisons indicated a significant increase of amplitude by the 6 mg/kg/day dose over that observed in the vehicle group (Dunnett's $t=2.58$, $p<0.05$). There was no effect of (+)-amphetamine on the phase of the circadian rhythm [Phase

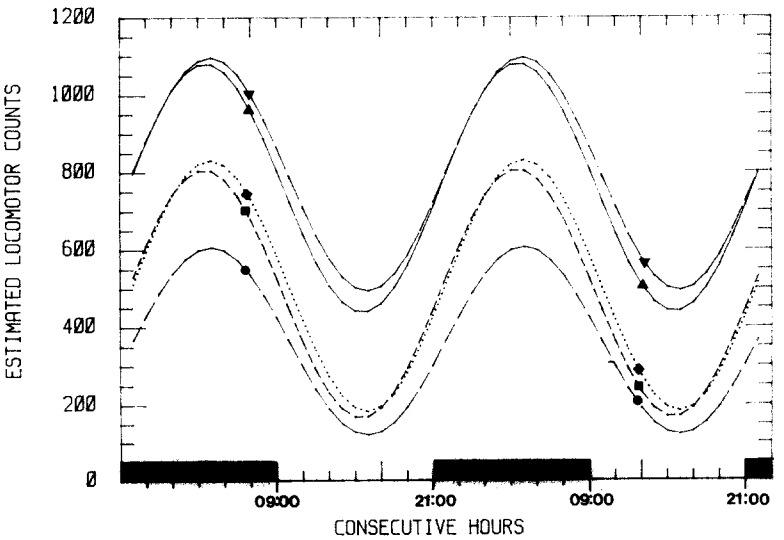

FIG. 4. Double plots of the effects of a series of doses of (+)-amphetamine on cosinor functions of the 24-hr rhythm in photobeam beam interruptions. The dark bars on the abscissa depict the periods when the lights were off. Locomotor counts were estimated by the cosinor procedure, from values for the phase, amplitude and mesor derived by spectral frequency analysis of the raw data. No effects on phases of the rhythms were noted with any dose. (+)-Amphetamine doses (mg/kg/day): vehicle (circle symbols and long dash lines), 2.0 (square symbols and short dash lines), 6.0 (diamond symbols and dotted lines), 10.0 (inverted triangle symbols and long dash lines) and 20.0 (triangle symbols and solid lines).

means ± SEM, in radians: 4.74 ± 0.03 (vehicle), 4.87 ± 0.06 (2 mg/kg/day), 4.73 ± 0.08 (6 mg/kg/day), 4.73 ± 0.09 (10 mg/kg/day), 4.83 ± 0.17 (20 mg/kg/day); $F(4,31) = 0.5, p > 0.1$]. While the cosinor functions were determined for all 15 days of drug treatment, the functions are basically the same if the first 4 days are excluded, as was done in a previous report (34).

DISCUSSION

These experiments indicate that there is a dose dependency in the development of daytime tolerance to the locomotor stimulant actions of (+)-amphetamine. A sustained tolerance does not occur to the locomotor stimulant effects of any of the doses during the night. Sustained daytime tolerance occurs to the locomotor stimulant effects of 2 and 6 mg/kg/day of (+)-amphetamine (0.083 and 0.25 mg/kg/hr), but not to 10 and 20 mg/kg/day (0.416 and 0.83 mg/kg/hr). Both diurnal and nocturnal activity levels were more variable across days in the groups receiving the two higher doses, perhaps reflecting interactions with stereotyped behaviors other than locomotion.

Circadian rhythm analysis revealed that the two highest doses significantly increased the mesor (overall mean of activity counts), but had no effects on amplitude or phase of the cycle. This is different from continuous administration of PHNO, a D2 agonist, which increases both mesor and amplitude of activity rhythms, reflecting daytime tolerance and nocturnal sensitization (34). Increases in the mesor by the lower doses of (+)-amphetamine were not significant; both lower doses increased the amplitude of the activity rhythm but only the 6 mg/kg/day dose increased it significantly. The daytime tolerance to the lowest dose of (+)-amphetamine was temporarily reversed by arousal stimuli, as with PHNO. No such effect was noted with the 6 mg/kg dose. The basic pattern of the effects of the low doses on the circadian rhythm of locomotor activity was therefore similar to that observed in the previous experiments with PHNO, but differed in degree (see Fig. 4). One major difference was that neither of the two lower doses of (+)-amphetamine produced nocturnal sensitization, as does PHNO.

The effects of the first few days of treatment in the present experiments are very similar to a previous report of 4 days of continuous subcutaneous infusions of (+)-amphetamine via electric infusion pumps (13). As in that report, the medium dose produced greater activity during the light than the dark on the second day, and the low dose in that study also resulted in daytime tolerance by the second day. However, the disruption of the circadian rhythm observed in both studies over the first days with the medium dose was not maintained with the longer treatment time in the present study. (+)-Amphetamine did not affect the phase of the circadian rhythms, while it has been reported that chronic administration of methamphetamine does (14). However, methamphetamine was administered via drinking water in this report. Circadian rhythms in drinking would determine the patterns of methamphetamine plasma levels, making it difficult to interpret such results in terms of direct methamphetamine actions on circadian rhythms.

The similarity of the effects of low but not higher doses of (+)-amphetamine to those of a selective D2 agonist suggest the possibility that low doses of sustained administration of (+)-amphetamine may be more efficacious at activating D2 than D1 receptors. Such a result may occur by down-regulation of D1 receptors. Recently, it was shown that a D1 selective agonist (SKF 38393), but not a selective D2 agonist (PHNO), produces an anorectic effect in rats (22). It was concluded that the anorectic effects of psychomotor stimulants may, therefore, depend upon D1 receptor stimulation. No changes in body weights have been produced by continuous infusions of PHNO in a number of experiments similar to the one described in the present paper. The present observation that the two lowest doses of (+)-amphetamine did not produce decreases in weight gain is indirect evidence for the view that these doses of (+)-amphetamine do not affect D1 receptors sufficiently to produce anorexia. The dose-dependent decrease in body weight gain with the higher doses is consistent with increasing activation of D1 receptors. Thus, the body weight data are consistent with the view that increased synaptic DA produced by lower doses of continuously administered (+)-amphetamine may preferentially bind to D2 receptors.

The highest two doses are close to doses of continuously infused (+)-amphetamine (SC) to which tolerance of motor stereotypies (assessed by rating scales) occurs over a seven day period (27). Indeed, casual observations indicate that rats receiving the highest dose of (+)-amphetamine in the present experiment exhibited intense focussed sniffing, licking and gnawing over the first few days, but not on subsequent days. This group displayed reductions in photobeam interruptions on the first day and during the second and third nights after pump implants. We believe this reflects the presence of intense focussed stereotyped behaviors on the basis of informal daytime observations. Experiments are currently in progress utilizing infrared-sensitive cameras to more rigorously examine this issue. It is quite possible that the progressive increase in locomotor activity observed in this group after the first few days and nights may therefore be a function of development of tolerance to these oral stereotypy effects, rather than to a "sensitization" of locomotor activity. Consistent with this hypothesis is the report that chronic infusion of 15 mg/kg/day of (+)-amphetamine initially produces intense focussed oral stereotypies, but that these stereotypies decline after 3 or 4 days, with an increase in locomotion corresponding to this decline (11). Whether such an explanation can account for the more gradual increase in the nocturnal activity of the group receiving 10 mg/kg/day awaits the conclusion of experiments in which such stereotypies are recorded during the night.

Tolerance to the motor stereotypy effects of continuous (+)-amphetamine have been related to neurotoxic effects on DA terminals in the caudate nucleus or decreases in levels of DA and its acid metabolites (6, 7, 28). Notably, these effects do not appear to occur in the nucleus accumbens (5, 6, 11), a region thought to be primarily responsible for the locomotor stimulant effects of (+)-amphetamine (17). This is consistent with the present observations of lack of tolerance developing to the highest two doses of (+)-amphetamine during any period of the light/dark cycle. Furthermore, that daytime tolerance does not occur to the locomotor stimulant actions of these doses is consistent with the hypothesis that tolerance is dependent upon loss of activation of D1 receptors. Of course, this is highly speculative; it remains to be established whether or not daytime effects of the two lower doses is consistent with this hypothesis. We are invoking a post hoc receptor selectivity of low dose (+)-amphetamine hypothesis based on similarities of effects to those produced by a selective D2 agonist and lack of effects similar to those produced by a D1 agonist. If this is not verified by future experiments, then the low dose (+)-amphetamine results, which we currently believe are "the exceptions that prove the rule," will not be consistent with a D1 receptor hypothesis of daytime tolerance to amphetamine's locomotor stimulation.

There is a biphasic daytime effect of the 10 mg/kg/day dose which strongly resembles a similar daytime biphasic effect of continuous DA infusion into the nucleus accumbens (3,4). Costall *et al.* observed peaks of activity on Days 3 and 10–11 of treatment, and troughs on Days 7 and 8. The 10 mg/kg/day group exhibited peaks on Days 2 and 10, with the intervening trough occurring on Days 7 and 8. The similarity of the biphasic effects of both systemically administered (+)-amphetamine and intra-accumbens

infusions of DA supports the view of increasing *regional* selectivity of amphetamine's actions (in favour of the nucleus accumbens) with decreasing dose.

In summary, the present findings indicate that tolerance to the locomotor stimulant actions of (+)-amphetamine is both dose- and light/dark cycle-dependent. Only lower doses result in tolerance, and only during the day. Locomotor stimulation remains high from Day 4 to Day 15 of drug treatments with high doses, and during the nights with low doses. However, nocturnal sensitization of locomotor effects of (+)-amphetamine was not observed. Thus, continuous administration of (+)-amphetamine does not produce both behavioural tolerance and sensitization, as does a selective DA D2 agonist. With this exception, effects of the low doses are roughly similar to those observed with continuous administration of a selective D2 agonist. This similarity suggests that DA released by continuous administration of low doses of (+)-amphetamine preferentially binds to DA D2 receptors in vivo. Lack of effects of the two lower doses on decreasing body weights is consistent with this hypothesis, as it has been previously shown that D1- but not D2-selective agonists induce anorexia (22). An intermediate dose of (+)-amphetamine produces daytime locomotor effects remarkably similar to a biphasic daytime effect produced with intra-accumbens infusions of DA (4), and this similarity suggests that intermediate doses of (+)-amphetamine may exhibit relative selectivity for the mesolimbic systems, at least during the day. Finally, the present results provide indirect support for previous evidence that DA-releasing terminals in the nucleus accumbens are resistant to neurotoxic actions of chronic, continuously infused (+)-amphetamine.

These results have certain implications for animal models of stimulant psychosis. Since the effects of some doses of (+)-amphetamine depend upon the light-dark cycle, perhaps it would be more reasonable to assess these effects during the night when animals would normally be awake, if generalizations to the human situation are to be made. Secondly, if the mesolimbic brain regions are those involved in stimulant psychosis, then perhaps amphetamine neurotoxicity is an inadequate model of psychosis, as suggested by Robinson and Becker (31). Finally, it may be fruitful to further investigate the differences between continuous and intermittent administration of relatively low doses of stimulants. An understanding of the mechanisms underlying these differences may provide insight into the underlying mechanism of stimulant psychosis.

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