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Amphetamine Cumulation and Tolerance Development: Concurrent and Opposing Phenomena

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SPARBER, S. B. AND L. H. FOSSOM. *Amphetamine cumulation and tolerance development: Concurrent and opposing phenomena.* PHARMACOL BIOCHEM BEHAV 20(3) 415–424, 1984.—Whether diminished or augmented behavioral effects are observed after repeated amphetamine administration may reflect the relative balance between tolerance and drug cumulation. To investigate this, we measured the distribution of d-amphetamine in various tissues and its effects on performance of a conditioned behavior after acute or chronic treatment. Rats trained to lever press under a fixed ratio 5 schedule for food-reinforcement were tested daily for 4 min epochs in each of 6 consecutive hours. After responding was stable, animals were injected for 16 days with saline or 1.0, 2.5 or 5.0 mg ^{3}H-d-amphetamine sulfate/kg IP 15 min before the second daily behavioral epoch. On the 17th day, animals which had been receiving ^{3}H-d-amphetamine were given their usual dose and those which had been receiving saline were given one of the doses of ^{3}H-d-amphetamine; all animals were decapitated approximately 2¼ hours after this final injection, immediately after the 4th behavioral epoch. Brain, heart, muscle, epididymal fat, and kidney were removed for subsequent analysis of unchanged ^{3}H-d-amphetamine. The experiment was carried out in two phases, 3½ months apart, which inadvertently resulted in shipment of rats from different buildings on the supplier's campus. Acute treatment produced dose-related effects on operant responding, the lowest dose increasing responding and the highest dose suppressing it. Chronic injection of the highest dose of d-amphetamine resulted in significant attenuation of its acute suppressant effect. Additionally, chronic treatment suppressed responding of rats 23¼ hours after injection (i.e., before the subsequent daily injection). Tissue levels of d-amphetamine were dose related and d-amphetamine cumulated after chronic treatment with the highest dose. When d-amphetamine was administered acutely, the behavioral effect immediately before decapitation was highly correlated with the concentration of d-amphetamine in brain and in heart. This was not the case after chronic treatment, since rats given the higher doses showed less behavioral effect than would have been predicted from the concentrations of d-amphetamine in their tissues. Besides evidence of tolerance and cumulation of drug in one or more tissues, a significant phase or colony difference emerged, which could have been due to seasonal or other factors. Additional, different experiments, performed concurrently on a new shipment of rats from each colony, allowed us to conclude that the original observations of phase differences were not due to seasonal differences or chance. Significant differences between the colonies emerged in both the rate of acquisition of an autoshaped behavior and its resistance to disruption by d-amphetamine. This demonstration of tolerance in the presence of cumulated d-amphetamine and colony differences is discussed in light of the equivocal nature of the literature on effects of repeated d-amphetamine administration.

Amphetamine Disposition Tolerance Operant Autoshaped behavior

d-AMPHETAMINE has a wide range of behavioral actions (for Review see [24]). At low to moderate doses (i.e., less than 2.5 mg/kg) it increases unconditioned motor activity in rats. Locomotor activity is generally attenuated at higher doses when it is replaced by stereotyped behaviors. Low doses (i.e., less than 0.5 mg/kg) generally increase conditioned responding emitted at low to intermediate rates, while moderate doses (i.e., 0.5–2.5 mg/kg) increase or progressively decrease responding, depending upon the baseline response rate, the schedule maintaining the behavior [10] and the controlling consequence [2]. Higher doses generally completely disrupt schedule controlled responding. Repeated administration can result in apparent tolerance, augmentation or no change in the effect of subsequent injections. Increased motor activity has usually been unchanged with repeated administration, however occasional reports of apparent augmentation [35, 36, 44] or tolerance [3, 18] occur. These have not been entirely convincing, since decreases in motor activity occur at higher acute doses when emergent stereotypies prevent its expression. Studies on stereotyped behaviors have generally failed to show tolerance or have demonstrated augmentation [7, 22, 29, 35]. In contrast, partial or complete tolerance has often been observed for the disruptive effects of d-amphetamine on operant conditioned responding [4, 5, 6, 17, 41, 45]. In general, tolerance and augmentation are seen with different behaviors and these different behaviors are often studied using different doses of d-amphetamine. Augmentation is usually observed for

stereotypies caused by high doses and tolerance develops to the disruption of conditioned behaviors caused by moderate doses.

Conditioning factors have been implicated for both augmentation and tolerance. Increases in motor activity or stereotypies have been reported to become conditioned to stimuli associated with drug treatment [12, 31, 44] and this may partially account for the augmented effects seen with subsequent injections. Conditioning factors may also have an impact on tolerance to operant behavioral disruption. Tolerance did not develop if subjects were not allowed to perform the operant behavior after each injection [5,6], suggesting that exposure to repreated d-amphetamine was not sufficient to produce tolerance and that some (repeated) interaction between drug administration and the behavioral response or the consequences of responding was necessary. As suggested above, another factor which may be important in determining the outcome of chronic treatment is cumulation of drug. Repeated administration of d-amphetamine alters levels of d-amphetamine in various tissues [23,38], which may interact with stress to produce a much greater behavioral effect than either the stress or drug alone [8]. Therefore, the effect of repeated d-amphetamine administration may depend upon several factors, including the behavior being measured, environmental factors, the dose, as well as route and schedule of administration, conditioning effects, and factors relating to the disposition and/or cumulation of d-amphetamine.

The present study investigates the involvement of cumulation of drug, in development and expression of tolerance or augmentation to d-amphetamine. Acute and chronic administration of a range of d-amphetamine doses (1–5 mg/kg) are investigated. A single behavior was used to measure the effects of all doses, rather than confounding the results and their interpretation by using different behaviors for different doses. We chose fixed ratio 5, food reinforced operant lever pressing by rats, since it generates lower overall response rates than higher fixed ratio schedules and would consequently allow us to detect both rate-increasing and rate-decreasing effects for low and high doses of d-amphetamine. Previous experience indicated low doses of d-amphetamine alters performance soon after administration, behavior returning to baseline within 1–2 hr. Higher doses completely suppress operant behavior for 1–2 hours, resulting in floor effects. This makes it difficult to compare behavioral alterations induced by a range of doses administered acutely or chronically, if the standard procedure of studying operant behavior for 1/2 to 2 hr after drug treatment is followed. Therefore, performance was sampled in 4 min epochs hourly for 6 consecutive hours. This method of sampling short behavioral epochs over several hours has provided a reliable measure of drug effects on operant responding during a long time span [13,38], while avoiding satiation encountered with long continuous sessions. In the present study, performance was sampled once (3/4 hr) before injection and 5 times, at hourly intervals, beginning 1/4 hr after injection. Additionally, concentrations of d-amphetamine in various tissues were determined, allowing the comparison of concentration-response relationships after acute and chronic treatment. Because of d-amphetamine cumulation, it was predicted that tolerance to operant behavioral suppression might either be masked or actually more pronounced if the tissue concentration, especially in brain or heart, were considered. Increased d-amphetamine tissue levels, due to cumulation with chronic administration, might interfere with the behavioral expression of tolerance, but the behavioral effect would still be less than would be predicted from the d-amphetamine concentration in tissue. Alternatively, if augmentation of the acute behavioral response occurred after repreated administration, it was predicted that elevated tissue levels might at least partially account for this.

EXPERIMENT 1

METHOD

Subjects

Subjects were 24 male Sprague-Dawley rats (Holtzman, Madison, WI). Because of logistics the experiment was done in 2 phases, 3 1/2 months apart, with 2 animals from each of the 6 treatment groups in each phase. Animals in the 2 phases were received in different shipments (and, as it turned out, from different colonies from the same supplier) but were matched for age (2 1/4 months old) and body weights (phase 1: 342±5 g; phase 2: 344±4 g; Mean±SEM). Animals were housed in individual hanging cages in a room with a 12 hour light/dark cycle (lights on 0800–2000 hr) maintained at 25°C and 50% humidity.

Apparatus and Procedure

Animals were gradually food-deprived to 80% of their free-feeding weights. They were trained to depress a lever for reinforcement with 45 mg food pellets (P. J. Noyes, Lancaster, NH) in a standard rodent operant chamber contained within a sound- and light-attenuating enclosure (BRS/LVE, Beltsville, MD). Schedule contingencies were controlled and data were collected and reduced by a minicomputer (Nova 2, Data General Corporation, Southboro, MA) interfaced with Interact hard- and software (BRS/LVE, Beltsville, MD). Cumulative recordings (Gerbrands Corporation, Arlington, MA) were also obtained. The number of lever presses required for reinforcement was gradually increased from 1 to 5 (fixed-ratio 5, FR 5). Animals were allowed to respond for one 4 min epoch each hour for 6 consecutive hours (0900–1500 hr) each day. Between epochs they were removed from the chamber and put back in their home cages, where tap water was available ad lib. Rats were tested daily for 2–3 weeks during which time they were habituated to IP injection with saline (1 ml/kg) 15 min before the second epoch. Baseline responding was determined for 5 days before drug treatment was begun (Table 1). Based on these control rates, animals were randomly assigned to 6 treatment groups, balanced for average response rates. For the next 16 days all animals were injected IP with either d-amphetamine or saline 15 min before the second behavioral epoch: one group received 1.0 mg ^{3}H-d-amphetamine/kg, another group received 2.5 mg ^{3}H-d-amphetamine/kg, a third group received 5.0 mg ^{3}H-d-amphetamine/kg, and the remaining 3 groups received saline (1 ml/kg). On the 17th day, animals which had been receiving d-amphetamine received their usual dose and each group that had been receiving saline was injected with one of the 3 doses of d-amphetamine. All animals were killed by decapitation immediately following the fourth behavioral epoch, and brain, heart, thigh muscle, epididymal fat and kidney were saved for subsequent analysis of ^{3}H-d-amphetamine.

TABLE 1
SUCCESIVE BEHAVIORAL EPOCHS

	1	2	3	4	5	6
Phase 1	54 ± 1.7	56 ± 1.9	56 ± 2.0	56 ± 2.0	54 ± 1.7	53 ± 1.7
Phase 2	56 ± 3.1	60 ± 3.7	59 ± 3.1	58 ± 3.4	58 ± 3.1	57 ± 3.2

Baseline fixed ratio 5 responding across 6 epochs, sampled for 4 min at hourly intervals. Values represent responses per minute averaged for 5 control days (Mean ± SEM, for 12 animals in each phase).

Drugs

Tritium labelled d-amphetamine sulfate (d-^{3}H(G)-amphetamine, New England Nuclear, Boston, MA) was combined with d-amphetamine sulfate (Sigma, St. Louis, MO) and dissolved in isotonic saline daily. The same amount of tritium (5 μCi/kg) was injected with each dose. Injections were always intraperitoneal in a volume of 1 ml per kg body weight. Doses are given for the salt.

Analysis of ^{3}H-d-Amphetamine

d-Amphetamine content of the various tissues was estimated as previously described [39]. Briefly, ^{3}H-d-amphetamine was extracted from tissue homogenate into benzene at a basic pH. It was then extracted from the benzene phase into formic acid. The amount of radioactivity recovered in an aliquot of the formic acid was corrected for recovery of ^{3}H-d-amphetamine carried through the extraction (89±3%, Mean±SD, n=10).

Data Analysis

The average response rates for each animal during each 4 min behavioral epoch, determined for the 5 days prior to the start of the experiment, served as control baselines. The response rate for each animal during each behavioral epoch during the experiment was expressed as a percentage of its appropriate baseline rate.

A 3-way analysis of variance (ANOVA) was performed on the data for each of the 4 behavioral epochs from the final day of the study, with dose (1.0, 2.5 or 5.0 mg d-amphetamine/kg), treatment (acute or chronic) and phase (first or second) as grouping factors. A least significant difference (LSD) test was used to further characterize significant effects [47]. Concentrations of d-amphetamine in the various tissues were similarly analyzed by 3-way ANOVA and LSD tests. Significance was determined by an alpha level of 0.05.

Least-squares linear regression analyses were performed to relate the behavioral response immediately before decapitation to the concentration of d-amphetamine in brain or heart after acute or chronic treatment. Where appropriate the 95% prediction limits were also calculated.

RESULTS

Behavioral Effects

Figure 1 shows data for the effects on FR 5 responding for

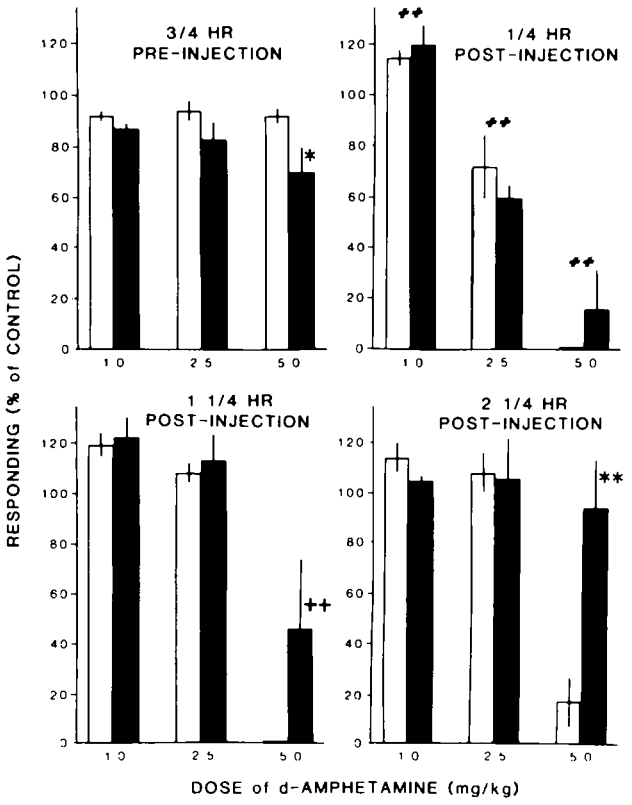

FIG. 1. Effects of acute and chronic administration of 1.0, 2.5 and 5.0 mg d-amphetamine/kg IP on FR 5 operant responding. Rats which received d-amphetamine acutely (open bars) had received saline (1 ml/kg IP) on the previous 16 days. Rats in the chronically treated groups (solid bars) received d-amphetamine daily for 17 days. Behavior was sampled in 4 min epochs before and after d-amphetamine injection. Responding in each epoch is expressed as a percentage of control responding for that epoch, determined for 5 days prior to saline or d-amphetamine administration. Values are Mean±SEM, for 4 rats in each group. *$p<0.05$, **$p<0.01$, chronic compared to acute treatment; ++$p<0.01$, chronic compared to acute treatment, for rats in phase 2 only; ##$p<0.01$, compared with next dose, collapsed across treatments (2-tailed LSD test).

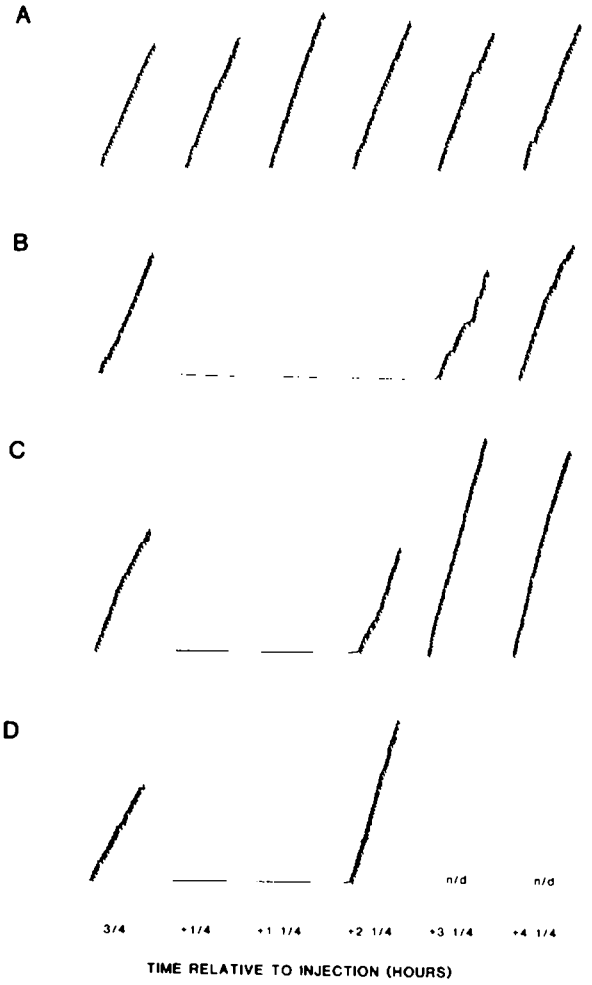

FIG. 2. Cumulative recordings of FR 5 operant behavior emitted by rat No. 8, treated chronically with 5.0 mg d-amphetamine/kg IP for 17 days. Responding during each of 6 hourly 4 min epochs is depicted for 4 days of treatment. Panel A shows control responding with saline injected 15 min before the second epoch. Panels B, C, and D show the first, sixteenth, and seventeenth (final) exposures to d-amphetamine, respectively. Response rates are indicated by the slopes of the recordings. Diagonal hatches indicate delivery of reinforcers. Rats were killed after the fourth epoch on day 17, therefore subsequent epochs are not depicted (n/d).

rats treated acutely or chronically for 17 days with 1.0, 2.5 or 5.0 mg d-amphetamine/kg. Analysis of variance demonstrated only a main effect of dose, $F(2,12)=71.11$, $p<0.005$, 1/4 hour after d-amphetamine administration with no difference between acute and chronic treatments at this time. Responding was increased at the lowest dose, with 6 of the 8 rats responding at rates more than 2 standard deviations above their individual baselines. Responding was decreased in a dose-related manner at the higher doses.

Analysis of variance of the data obtained 1 1/4 hours after d-amphetamine administration showed a significant 3 way interaction of dose, treatment and phase, $F(2,12)=9.19$, $p<0.005$. This was due to apparent tolerance to the behavior suppression at the high dose, but only for animals in the second phase. This phase difference in the extent of tolerance development was also reflected in the tissue levels of d-amphetamine (see below).

Two and one quarter hours after d-amphetamine administration there were significant dose, $F(2,12)=13.59$, $p<0.005$, and treatment, $F(1,12)=5.93$, $p<0.05$, effects, as well as, a dose by treatment interaction, $F(2,12)=9.00$, $p<0.005$. Tolerance was evident for all rats at the highest dose (regardless of phase). Rats treated acutely were suppressed to 17% of control response rates while those treated chronically were responding at 96% of control.

For the behavioral session 1/4 hour before d-amphetamine administration (i.e., 23 1/4 hours after the 16th administration in animals receiving d-amphetamine chronically), there was a significant treatment effect, $F(1,12)=8.83$, $p<0.05$, with animals treated chronically showing suppression, especially at the high dose.

Some of the effects described above are illustrated in Fig. 2 by cumulative recordings obtained for a rat which received the highest dose of d-amphetamine chronically. Panel A shows control responding by that animal; injection of saline 15 min before the second 4 min epoch did not affect subsequent responding. The first administration of 5.0 mg d-amphetamine/kg (15 min before the second epoch; Panel B) completely suppressed responding during the following 3 epochs; responding had partially returned by 3 1/4 hr after injection. On the sixteenth day of repeated administration (Panel C) responding had returned by 2 1/4 hr after injection. The increased responding 3 1/4 and 4 1/4 hr after injection, which is apparent for this rat on day 16 of treatment (panel C), was seen for 3 of the 4 rats which received the high dose chronically. Their responding after the first injection of d-amphetamine was less than or not different from baseline (mean + 2SD) but after repeated treatment their responding in these epochs was elevated (more than 2SD) above baseline. The shortening of the duration of behavioral suppression, (i.e., tolerance) was also evident on the seventeenth day of injections (Panel D), when animals were killed immediately after the fourth behavioral epoch (approximately 2 1/4 hr after injection).

Tissue Levels of d-Amphetamine

Concentrations of d-amphetamine in the various tissues after acute and chronic treatment are presented in Fig. 3. Results for the 2 phases of the study are presented separately since ANOVA showed a significant phase by dose by treatment interaction for 3 tissues, $F(2,12)=13.14$, 6.43, and 5.86 for brain, heart and kidney, respectively; $p<0.02$ for each. Inspection of the data shows that tissue concentrations are dose-related after acute administration regardless of phase. After chronic administration of the highest dose, d-amphetamine cumulated to a significant extent in brain, heart, muscle, fat and kidney from animals in the first phase, but only in fat of those from the second phase. The cumulation of d-amphetamine in tissues of rats from the first phase and not in those from the second phase is consistent with the observation that responding returned 1 hr earlier in animals from the second phase treated chronically with the highest dose (*vide supra*). Concentrations after acute treatment were not different for the 2 phases, except in fat, where animals in the second phase receiving the highest dose had lower concentrations than those in the first phase.

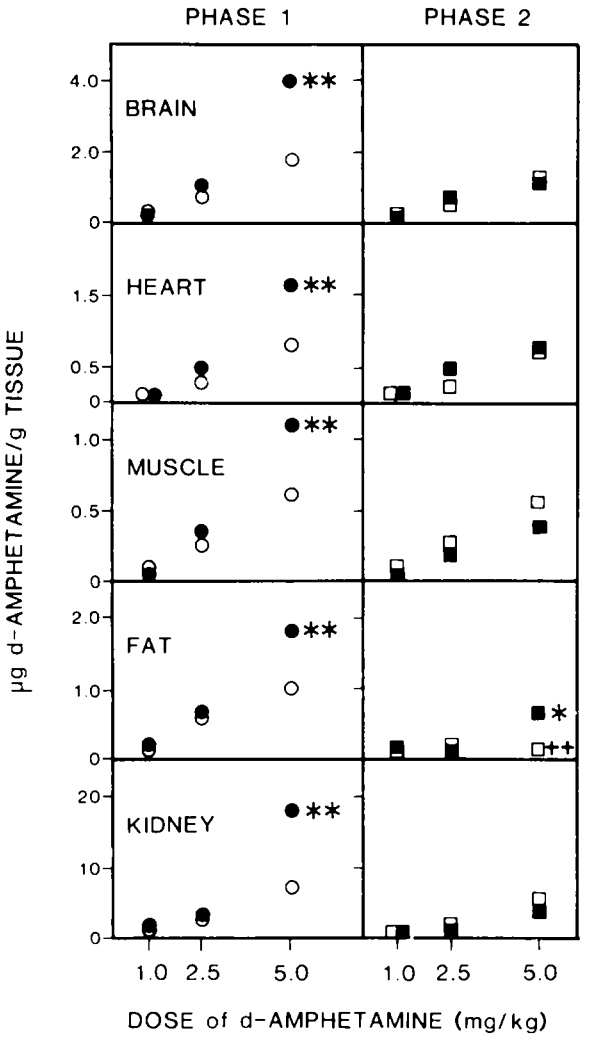

FIG. 3. Tissue levels of d-amphetamine after acute or chronic treatment. Circles depict data from the first phase of the experiment, squares from the second phase. Open symbols denote acute treatment, solid symbols denote chronic treatment. Each symbol represents a mean value for 2 rats. $*p<0.05$, $**p<0.01$, chronic compared to acute treatment; $++p<0.01$, phase 2 compared to phase 1 (2-tailed LSD test).

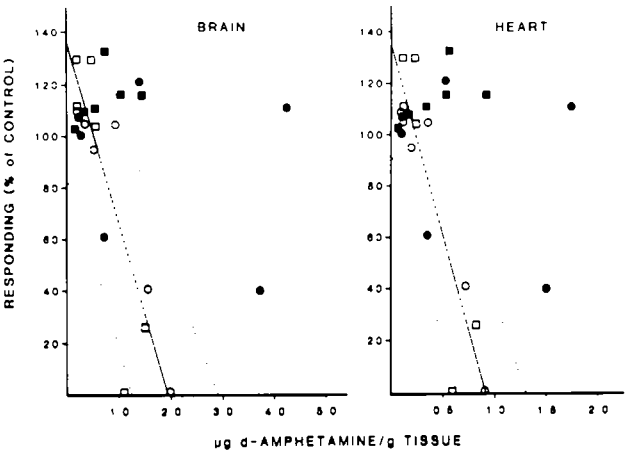

FIG. 4. Correlation between brain or heart concentration of d-amphetamine and behavioral response after acute administration and lack of correlation after chronic treatment. The behavioral response immediately before killing the rats is plotted as a function of concentration of d-amphetamine in tissue for each subject. Circles depict data from the first phase of the experiment, squares that form the second phase; open symbols represent rats treated acutely, solid symbols those treated chronically. After acute administration, the behavioral response is highly correlated with brain levels ($y=-0.699\times+1.362$, $R_{10}=-0.870$, $p<0.001$) and with heart levels ($y=-1.467\times+1.350$, $R_{10}=-0.903$, $p<0.001$). Dotted lines indicate the 95% prediction limits for behavioral response regressed against tissue concentration after acute treatment.

Behavior as a Function of Brain or Heart Concentration

After acute administration of d-amphetamine, the behavioral effect immediately prior to decapitation (i.e., 2¼ hours after administration) is highly correlated with concentration of d-amphetamine in brain or in heart. Linear correlation analyses were not performed on the data from other tissues because it was felt that brain and heart were representative target tissues probably responsible for behavioral effects of d-amphetamine and indicative of the most important relationship between drug concentration and effect. Figure 4, reveals that the linear relation between tissue concentration and behavioral effect, which is so evident after acute administration, is lacking after chronic treatment (brain: $R(10)=-0.348$, $p>0.1$; heart: $R(10)=-0.272$, $p>0.1$). Additionally, the points from data for all of the animals which received the highest dose and 2 of the 4 animals which received the median dose chronically lie to the right of the 95% confidence limits for the behavioral response predicted from either tissue level of d-amphetamine. Thus, the behavioral effect is actually less than would be predicted from the brain or heart levels, as well as being less than would be expected at the dose administered (Fig. 1). It is of particular interest that tolerance to d-amphetamine-induced behavioral disruption was evident even in the 2 rats in phase 1 which had greatly elevated tissue levels of d-amphetamine after chronic treatment.

EXPERIMENT 2

The first experiment reported herein was carried out in 2 phases because of its size, duration and complexity. Care was taken to control for confounding variables which might otherwise influence the outcome. Nevertheless, during the course of the behavioral component it became apparent that responsiveness to the behavioral effects of d-amphetamine differed between subjects in the two phases. This was subsequently borne out by ANOVA of both the behavioral and tissue concentration data, which included *phase* as one of the factors. Since significant phase differences emerged, we could not be certain as to their origin, be they seasonal, genetic, ontogenetic, etc. However, we felt it was important to ascertain if genetic and/or ontogenetic factors also were important determinants of the outcome of experiments of this type, especially if there was no reason to believe the

same supplier was not shipping "identical" rats. When we found out that the supplier shipped rats from one building (Colony 4) for phase 1 and shipped rats from another building (Colony 3) for phase 2, we chose to do a contemporaneous experiment using subjects from each colony. We felt that investment of time and effort for training and stabilizing 4 min epochs of FR behavior might not be necessary for determining if inherent differences in conditioned behavior and/or its sensitivity to disruption by d-amphetamine existed between the so-called "identical" colonies (i.e., phases). If we could establish that such differences existed without doing a chronic study or determining tissue concentrations, it would corroborate our initial observations and help us interpret the otherwise unexpected phase (colony) differences we observed in the first experiment. Additionally, if such differences emerged in an experiment which utilized a considerably different behavioral procedure, the robustness and generalizability of the importance of such factors would be strengthened. Accordingly, the second experiment utilized a modified autoshaping procedure wherein delivery of the reinforcing stimulus (food pellet) was under a random-time schedule associated with the retraction of a lever which previously emerged from behind the manipulandum panel; or contingent upon a lever-touch response, which caused immediate retraction of the lever and delivery of the food pellet. As such, we have chosen to refer to the procedure as an autoshaped operant but it has been referred to as a forward-pairing autoshaped procedure as well [1]. After acquisition and stabilization of this conditioned behavior at asymptotic levels, the rats were challenged with d-amphetamine to determine whether they were also differentially sensitive to the behavioral disruptive action of this drug. While the previous experiment, which suggested that this might be the case, was carried out on rats performing an operant maintained on a FR 5 schedule of reinforcement, the autoshape procedure, with pharmacological challenge utilized in this experiment has been demonstrated to detect behavioral teratogenic consequences of exposure to otherwise relatively nontoxic doses of methylmercury *in utero* [20]. Since we have previously shown this behavior to be more resistant to disruption than FR responding [37], we used a higher dose of d-amphetamine than those used in the previous study. We sampled behavior at approximately 2 hr after injection, the median time used in the previous study.

METHOD

Subjects

Ten adult (4 month old) male Sprague-Dawley rats from each of two colonies from which subjects were obtained for the previous experiment (Colonies 4 and 3, Holtzman, Madison, WI, as used in Phases 1 and 2 of Experiment 1, respectively) served as experimental subjects. They were individually housed and gradually reduced to 80% of their free-feeding weights. Their final weights ranged from 350 to 400 g. They were subsequently tested on three consecutive days for acquisition of an autoshaped operant response.

Apparatus

A standard rodent operant chamber was equipped with a retractable lever and 8 cm wide metal strips near the top of 2 adjacent walls as described elsewhere [20,37]. Touches by the rat which completed a circuit (less than 2.5 M Ohms resistance) between the grid floor of the chamber and either the lever or the strips were counted. It is problematic whether initial lever-touches are a reflection of horizontal exploratory activity or a combination of such random activity plus responses directed toward the lever as conditioning occurs. Rearing (strip-touching) is a measure of exploratory activity and tends to diminish as the rats habituate to the chamber, diminishing even more as they acquire and perform the autoshaped response, attending more and more to the lever or the hole it emerges from [27]. A computer (Nova 2, Data General Corporation, Southboro, MA) interfaced with Interact hardware and software (BRS/LVE, Beltsville, MD) controlled environmental contingencies and collected and reduced data.

Autoshaped Behavior

During autoshaping sessions, the lever was extended into the operant chamber an average of once each minute, the time between presentations ranging randomly from 30 to 90 sec. If the rat touched the extended lever, completing a circuit with the grid floor, the lever retracted and concurrently a 45 mg food pellet (BioServ, Frenchtown, NJ) was delivered. If the rat did not touch the lever, it was retracted after 15 sec and a food pellet was delivered. An autoshaping session consisted of 20 lever presentations. In addition to cumulative recordings of lever contacts (R. Gerbrands Co., Arlington, MA) during each session, a computer printout of latencies to respond to each lever presentation, total lever touches, and total strip touches were obtained.

Drugs

d-Amphetamine sulfate (Sigma, St. Louis, MO) was dissolved in isotonic saline to contain 7.5 mg d-amphetamine sulfate per ml. Injections were intraperitoneal in a volume of 1 ml per kg body weight.

RESULTS

Analyses of variance performed on data from the first 3 days of autoshape acquisition with colony (3 or 4) as the grouping factor showed significant effects of repeated testing on all variables (correct responses, latencies and strip touches) (Fig. 5). Animals made more correct responses, $F(2,36)=31.40$, $p<0.001$, with shorter latencies $F(2,36)=42.66$, $p<0.001$, indicating acquisition of the lever touch response. At the same time, rearing behavior (i.e., strip touching) diminished, $F(2,36)=4.47$, $p<0.025$. Consistent with our expectations, ANOVA also indicated a main effect of colony on all dependent variables, with rats from Colony 3 making more correct responses, $F(1,18)=4.56$, $p<0.05$, with shorter latencies, $F(1,18)=4.75$, $p<0.05$, and rearing less, $F(1,18)=6.76$, $p<0.025$. There was no significant interactions for any dependent variable.

Animals were allowed 14–16 additional daily sessions to stabilize on the autoshaped operant. The average of the last 5 days served as baseline rate for each animal (Table 2). Two rats (one from each colony) were dropped from further study because their autoshape performance was erratic. Rats from Colony 3, which had acquired the autoshape response more rapidly, had slightly longer latencies to respond than rats from Colony 4; however, all rats were responding to essentially all lever presentations at the time baselines were determined two weeks after initial training.

Rats from each colony were randomly assigned to two treatment groups. One group (5 rats from each colony) re-

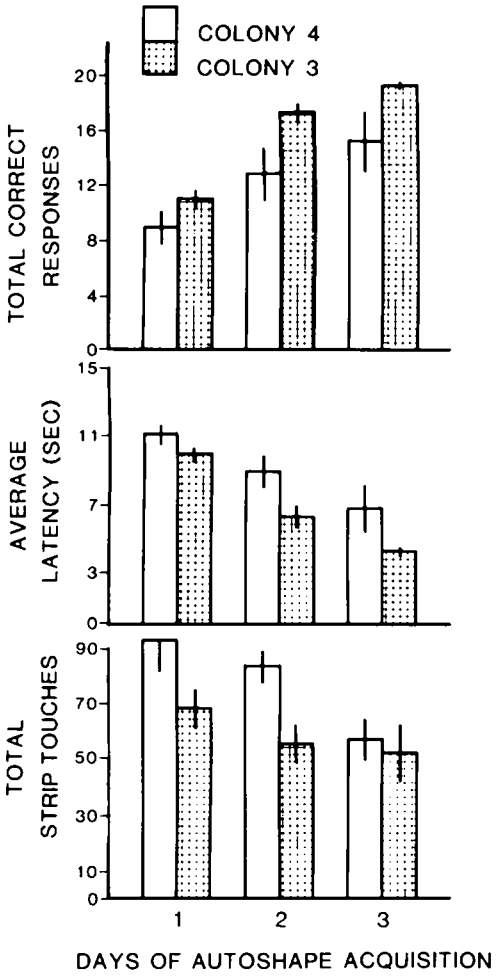

FIG. 5. Differential acquisition of an autoshaped operant by rats obtained from different, but "identical" colonies. Values are Mean±SEM for 10 rats from each colony. Although significant effects of repeated testing indicated subjects from both colonies learned the response, rats from colony 3 made significantly more correct responses, $F(1,18)=4.56$, $p<0.05$, with shorter latencies, $F(1,18)=4.75$, $p<0.05$, and less rearing, $F(1,18)=6.76$, $p<0.025$, than those of Colony 4.

TABLE 2
BASELINE RATES OF AUTOSHAPED BEHAVIOR

	Colony 4	Colony 3
Total Correct Responses	19.7 ± 0.1	19.5 ± 0.2
Average Latency (sec)	1.8 ± 0.2*	2.6 ± 0.3
Total Strip Touches	29.5 ± 5.8	22.5 ± 7.0

Values represent averages for 5 control days (Mean ± SEM, for 9 animals in each colony).
*$p<0.05$, Compared with colony 3, $F(1,16)=4.71$, ANOVA.

TABLE 3
COLONY DIFFERENCES IN EFFECTS OF ACUTELY ADMINISTERED d-AMPHETAMINE ON AN AUTOSHAPED OPERANT

	Colony 4		Colony 3	
	SAL	d-A	SAL	d-A
Total Correct Responses	19.2	5.0*	19.8	16.5
Average Latency (sec)	2.1	12.0*	2.4	4.3
Total Strips Touches	47.8	12.0	39.5	6.4

Values (Mean ± SEM) represent 4 animals from each colony given saline (SAL) and 5 animals from each colony given 7.5 mg d-amphetamine/kg IP approximately 2 hr before testing.
*$p<0.01$ compared with each other group (2-tailed LSD test).

ceived 7.5 mg d-amphetamine/kg IP one hour 50 minutes before the behavioral session; the other group (4 rats from each colony) received saline IP one hour 50 minutes before the behavioral session.

ANOVA's on total correct responses, average latency and total strip touches (expressed as percentage of baseline values), with colony (3 or 4) and treatment (saline or d-amphetamine) as grouping factors, now revealed colony differences in response to acute d-amphetamine (Table 3). Rats from Colony 4 were greatly affected by d-amphetamine. They responded to fewer lever presentations, $F(1,14)=4.90$, $p<0.05$, and had longer average latencies, $F(1,14)=6.04$, $p<0.05$, than rats from Colony 3 that received d-amphetamine (which were hardly affected) or rats from either colony which received saline. There were no significant effects on strip touching, although there was a tendency for strip touches to be lower after d-amphetamine, $F(1,14)=4.37$, $p<0.055$.

The results of this study demonstrate differences between rats of the same strain from a single supplier matched for sex, age, body weights and presumed to be identical. Differential acquisition of an autoshaped operant and differential responsiveness to acutely administered d-amphetamine were found, in spite of the similar baselines of this behavior when they were challenged with drug. Rats from Colony 4, which showed cumulation of d-amphetamine after chronic treatment in Experiment 1, were more sensitive to acutely administered d-amphetamine in Experiment 2.

DISCUSSION

This study was designed to measure effects of a broad dose range for d-amphetamine over several hours. These results show the initial effect (i.e., 15 min after injection) of acutely administered d-amphetamine on lever pressing maintained under an FR-5 food reinforcement schedule was dose-ralated; that 1 mg/kg increased responding and 2.5 mg/kg and 5.0 mg/kg suppressed responding in a dose related manner. Responding remained suppressed for at least 2 1/4 hr after the highest dose but had recovered by 3 1/4 and 4 1/4 hr (group data not presented). Two points require further explication. Firstly, the operant behavioral suppression as a measure of drug action was subject to a floor-effect and, 1/4 to 1 1/4 hr after the highest dose, the observed behavioral suppression was probably a conservative estimate of the

actual effect. Secondly, it is possible the lowest d-amphetamine dose would have produced a short-lived suppression of responding which was no longer apparent at the time of the first test epoch (i.e., 15 min after injection). This d-amphetamine dose has been previously shown to suppress FR 30 food maintained lever pressing within approximately 5–10 min of injection when the animal's behavior was continuously monitored [42]. d-Amphetamine-induced suppression can be considerably attenuated by returning the animal to its home cage for 10 min before behavioral testing of operant responding [42].

After repeated administration of d-amphetamine for 17 days, alterations in operant behavioral effects were still dose-related. However, the effect of repeated administration upon operant behavior also turned out to be dose-dependent. At the lowest dose, no change in the increased responding was observed; it was neither augmented nor attenuated. Similarly, no apparent difference in operant behavior was demonstrated between acute and chronic treatment with the median dose, although brain and heart concentrations in half the rats treated chronically were higher than would be expected from their behavior. Behavioral tolerance emerged for the highest dose with chronic administration. Tolerance was evidenced by a shortening of the duration of the operant behavioral suppression. A decreased response to this dose would most readily be seen as a shortening of duration rather than as a diminution of peak effect, since the peak effect after acute treatment was very likely greater than could be measured by the behavioral suppression at this dose (i.e., complete suppression resulting in a floor-effect). The duration of operant suppression has been reported to be directly related to the dose of d-amphetamine administered [4,30] and the shorter duration of suppression with the highest dose after repeated treatment indicates a lower-dose effect; that is, tolerance had developed. As mentioned in the Introduction, tolerance to d-amphetamine's effects on operant behavior has been previously reported but not under all conditions. It is possible that the mechanism for and/or rate of tolerance development (if at all) to the response-increasing action of d-amphetamine is different from the mechanism for and/or rate of tolerance development to the response-suppressing action of the drug. Schuster and coworkers [34] suggested tolerance develops to effects which interfere with accomplishment of schedule contingencies but not to effects which facilitate it. Our data support this theory inasmuch as we observed tolerance to the response suppressing effects of the highest dose but no tolerance to the response-facilitating effects of the lowest dose.

Repeated administration of d-amphetamine also produced progressive suppression of responding 23¼ hr after injection, which was significant for the highest dose. This "baseline shift" could be due to some sort of conditioned aversion because the daily dose of d-amphetamine was administered shortly after this epoch [15]. d-Amphetamine administered immediately after daily operant sessions using fixed ratio or fixed interval schedules of reinforcement has been reported to suppress responding in subsequent daily sessions [11,43].

Alternatively, suppression 23¼ hours after the previous administration of d-amphetamine may be evidence of withdrawal. This interpretation is supported by the observation that repeated administration of the highest dose resulted in tolerance to the acute behavioral-suppressant effect 2¼ hours after injection and responding had completely recovered by 4¼ hours. Others have also reported a "withdrawal depression" syndrome after repeated administration of d-amphetamine to animals [46] or man [28,33]. If the baseline shift was the result of a residual effect upon chronic injection (conditioned aversion and/or drug cumulation), one should expect augmented FR behavioral suppression after daily injection, not tolerance. In previous research it has been demonstrated that FR operant behavior studied in a similar manner can be a sensitive indicator of spontaneous withdrawal [14] when drugs are administered chronically.

The determinations for relative distribution and amounts of d-amphetamine in various tissues after acute administration presented here agree well with those reported by others [9, 21, 23, 25]. d-Amphetamine can cumulate in tissue after repeated administration, with brain and heart levels increasing as much as 2-fold after 5.0 mg d-amphetamine/kg. Similar findings have also been reported by others, but following more frequent dosing schedules, Kuhn and Schanberg [23] found a 40% increase in brain levels and 80% increase in heart levels of d-amphetamine 12 hours after the last of 6 intraperitoneal injections of 5 mg d-amphetamine/kg given at 12-hour intervals. Similarly, Segal and coworkers [36] found a 50% increase in brain 3 hours after the last of 31 injections of 2.5 mg d-amphetamine (base)/kg given subcutaneously at 4-hour intervals. In contrast, cumulation has not always been observed. Lowered d-amphetamine levels in brain and unchanged levels in heart and kidney (though fat levels were increased) have been previously reported after 6 daily injections of 2.5 mg d-amphetamine (base)/kg subcutaneously [39]. In the present study no evidence of cumulation was found when 1.0 or 2.5 mg d-amphetamine/kg was administered intraperitoneally daily, for 17 days. Five mg/kg produced elevated levels of drug in all 5 tissues that were analyzed for one cohort of rats but some cumulation of unchanged d-amphetamine was only evident in fat (not brain, heart, muscle or kidney) for another cohort. The results of this study, while demonstrating a difference in cumulation between rats in the 2 phases, did not allow us to characterize the source of this variability.

More importantly, brain and heart levels of amphetamine correlated with behavioral response after acute administration, regardless of any individual or phase differences. This is not an unexpected finding, but it has not been so clearly demonstrated previously for operant behavior. Maickel and coworkers [25] determined brain concentrations and effects on several conditioned behaviors at several time intervals (from 0.5 to 8 hr) after acute administration of d-amphetamine over a 30-fold dose range. They found a broad range of concentrations of d-amphetamine in brain at different doses and times after injection. However, of their behavioral measures, only the food-reinforced portion of a discrimination procedure (with a shock maintained escape/avoidance as the other behavior) showed a large enough range of disruption by d-amphetamine (6–78%) to be used in a correlation analysis. Their data show that, at a given time after acute administration (specifically 0.5–3 hr), brain concentrations and suppression of operant responding for food reinforcement were directly related to the d-amphetamine dose, with higher doses producing higher brain concentrations and greater response suppression. However, because the dispositional and behavioral measurements were determined on different subjects, it would be unwise to speculate further regarding a functional relation between brain concentrations of drug and behavioral effects. Since behavioral manipulations can affect d-amphetamine disposition [26, 32, 39], in the current study the same sub-

jects were used for dispositional and behavioral determinations. Thus, the linear concentration-response relationships control for effects on d-amphetamine disposition produced by the behavioral procedure and at the same time probably reflect more closely the effects on operant responding related to d-amphetamine concentrations in tissue.

Although brain or heart d-amphetamine concentration and operant behavioral response were highly correlated after acute administration, a significant correlation was not evident after chronic treatment. After repeated administration of higher doses, the behavioral suppression was much less than would have been predicted from the levels of d-amphetamine found in brain or heart. Animals which showed diminished behavioral disruption after repeated administration of a given dose of d-amphetamine also showed a diminished behavioral effect (i.e., tolerance) based on tissue d-amphetamine levels, even when cumulation was evident. In fact, for all animals receiving the highest dose and half the animals receiving the median dose chronically, the behavioral response was much less than would have been predicted from brain or heart levels of d-amphetamine. Thus, tolerance to the operant behavioral suppressant effect of d-amphetamine was evident even in the face of elevated levels of d-amphetamine in target tissue. Increased tissue concentrations without evidence of enhanced behavioral effects or enhanced behavioral effects in the presence of tissue concentrations which are proportionately even more elevated should also be considered as evidence of tolerance.

The highest dose produced effects upon behavior after chronic injection which were varied and most interesting. It appears that the magnitude and duration of response suppression was great enough to induce behavioral tolerance to this effect and as tolerance developed, even in the face of significant cumulation in half of the subjects, there emerged significantly increased responding in epochs 3¼ or 4¼ hr after chronic injection in 3 of the 4 subjects, which was not evident after acute injection.

This phenomenon may be interpreted in several ways. Simply, it may be a rebound increase in responding after maximal suppression in earlier epochs which would have emerged after acute injection, had we studied them for more than 4¼ hr after injection. As tolerance to the response-suppressing action of d-amphetamine developed, the time-effect curve shifted to the left, unmasking this effect because it was now occurring while we studied the rats' behavior. Alternatively, the rate-increasing action of d-amphetamine on FR 5 behavior is incompatible with its rate-suppressing action and emerges as tolerance to the latter effect develops. This interpretation is somewhat akin to the concept discussed earlier whereby emergence of stereotypies is incompatible with locomotor behavior, thereby suppressing the latter.

In summary, the time course of the acute effects of rate-increasing and rate-decreasing doses of d-amphetamine on operant behavior were determined. Tolerance to the behavior-suppressant effect of d-amphetamine was demonstrated while failing to find tolerance to its behavior-increasing effects. d-Amphetamine cumulated in various tissues after repeated IP administration of the high dose, but not lower doses. This cumulation was not observed for all rats and a subsequent study suggested that genetic or ontogenetic differences between rats, as reflected in differences in acquisition of an autoshaped operant and in the behavioral response to acute treatment, may also be responsible for differences in drug cumulation after repeated administration. Tolerance was evident even in the presence of elevated tissue levels of d-amphetamine, but was even more obvious when these elevated levels were taken into account. The co-development and co-existence of tolerance and cumulated drug in target tissues and the relative balance of these two opposing phenomena may be responsible for tolerance as well as augmentation of a particular behavioral or physiological measurement after repeated administration. The confounding effects of these two opposing factors, as well as genetic and ontogenetic differences between subjects, may help to explain some of the diverse and apparently contradictory results reported in the literature.

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