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Repeated Binge Exposures to Amphetamine and Methamphetamine: Behavioral and Neurochemical Characterization[1]

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ABSTRACT

Stimulant psychosis and addiction are most commonly associated with repeated, high-dose binges or runs, typically preceded by a more intermittent pattern of stimulant abuse. We previously reported that rats exposed to an escalating dose-run pattern of amphetamine administration exhibited changes in their behavioral response profile that differed both qualitatively and quantitatively from the response to either acute or intermittent daily treatment. To determine the generality of these effects and characterize further the nature of the behavioral and neurochemical changes of this treatment, rats received single daily injections of amphetamine (2.5 or 4.0 mg/kg s.c.) or equimolar doses of methamphetamine, followed by multiple runs (four daily injections at 2-hr intervals) with the pretreatment dose. This treatment resulted in a unique behavioral profile, including a profound increase in the relative expression of locomotion *vs.* stereotypy. The markedly enhanced poststereotypy locomotor activation was characterized by repeated "burst"-like episodes of ambulation. The number of runs required for the emergence of this behavior was dose dependent and was similar for the two drugs except that with methamphetamine, there also was a marked prolongation of the poststereotypy locomotor response during run exposures. During runs, both drugs produced a decline in the caudate but not the nucleus accumbens microdialysate dopamine response, whereas only methamphetamine produced a decline in the serotonin response that was apparent in both regions. The possible relationship between these behavioral and neurochemical changes and their implications for high dose patterns of stimulant abuse are discussed.

Stimulant addiction and the induction of a paranoid-like psychosis are most commonly associated with a high-dose binge or run pattern of stimulant abuse (Angrist, 1994b; Davis and Schlemmer, 1980; Gawin and Khalsa, 1996), generally preceded by a relatively long-term, intermittent or escalating dose exposure (Angrist, 1987, 1994a, 1994b; Gawin, 1991; Gawin and Khalsa, 1996). Although numerous factors affect both the pattern and outcome of chronic stimulant intake, it has been suggested that an escalating dose-binge pattern of stimulant exposure in experimental animals might provide valuable insight into the behavioral and neurochemical alterations that occur most frequently during the course of high-dose stimulant abuse (Gawin and Khalsa, 1996; Schmidt *et al.*, 1985b; Segal and Kuczenski, 1997).

Observations over the past decade have suggested that repeated SDI of relatively high AMPH doses (≥ 4.0 mg/kg) result in an altered behavioral profile that includes elements of both tolerance and sensitization (Eichler *et al.*, 1980; Rebec and Segal, 1980; Segal, 1975; Segal *et al.*, 1995). Furthermore, with multiple daily injections, these changes became even more pronounced, and some qualitative alterations in the response also appeared to emerge primarily in the form of decreased variation in behavioral expression (Segal *et al.*, 1980).[2] Recently, we characterized these effects more systematically during the course of exposure to escalating doses followed by repeated runs (Segal and Kuczenski, 1997). For this study, we used the highest daily binge doses that, after an escalating dose pretreatment, did not produce any obvious physiological or behavioral toxicity under our experimental conditions [*i.e.,* four daily injections of AMPH (8.0 mg/kg) every 2 hr for ≤9 days.] The results revealed the emergence of a unique behavioral profile that was characterized by a pronounced increase in both the magnitude and duration of locomotor activation and a decrease in continuous stereotypy. Furthermore, when observations extended beyond the stereotypy phase, the animals appeared to be highly agitated

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[2] D. S. Segal and R. Kuczenski, unpublished observations.

ABBREVIATIONS: AMPH, amphetamine; METH, methamphetamine; DA, dopamine; NE, norepinephrine; 5-HT, serotonin; SDI, single daily injection(s); ICI, intercrossover interval; ANOVA, analysis of variance; AUC, area under the curve.

after multiple runs and engaged in burst-like episodes of locomotion, which is uncharacteristic of acute or repeated intermittent treatments.

In parallel studies, regional neurochemical analyses, using *in vivo* dialysis techniques, showed that the escalating dose-run treatment produced significantly different response profiles for DA, NE and 5-HT. Most notably, we observed a progressive decrease in caudate DA and 5-HT, both within and between successive runs. In contrast, the hippocampal NE response did not decline and, in fact, tended to increase during this treatment.

The present study was designed to confirm and extend these observations. The binge effects of lower AMPH binge doses (*i.e.,* 2.5 and 4.0 mg/kg) were characterized to determine whether the unique behavioral profile that we previously observed to occur in response to binge exposure is restricted to relatively high-doses. Similar findings with lower doses would extend the potential relevancy of these observations to a wider range of stimulant abuse patterns. The 2.5 mg/kg dose of AMPH was selected because it is the lowest dose that, under our experimental conditions, produces a multiphasic response profile, including a distinct, continuous phase of stereotypy. The 4.0 mg/kg dose was included both because the stereotypy it induces is qualitatively different from the lower dose (oral as opposed to repetitive head movements) and because its pattern of behavioral alteration with repeated SDI, unlike the lower dose, includes elements of both sensitization and tolerance (Segal *et al.*, 1995). Furthermore, to more closely simulate stimulant abuse patterns, exposure to both the pretreatment regimen and run phases were prolonged, and both phases were interrupted by drug-free periods (Gawin, 1991). In addition, to more accurately characterize the apparent qualitative changes in the locomotor response, our observational ratings were extended beyond the continuous stereotypy phase to include the entire poststereotypy response. Furthermore, to quantify the burst-like locomotion, rates of crossover activity were determined.

In addition to AMPH, the effects of binges with METH were also assessed to determine the generality of the AMPH-induced alterations. Although the two drugs have similar mechanisms of action, their acute neurochemical profiles have been shown to differ (Kuczenski *et al.*, 1995), particularly with respect to their relative effects on NE and 5-HT. Therefore, comparison of the responses to AMPH and METH might provide insight into the role of these transmitters in the behavioral profile that emerges with repeated run exposures.

The results of these studies indicated that the unique behavioral profile associated with the high-dose AMPH binges also occurred with lower doses of AMPH and METH. In addition, a number of differences were apparent between the drugs, most notably a marked prolongation of the locomotor response to METH during runs. These findings confirm and extend our original observations and provide further insight into possible underlying mechanisms.

Methods

Subjects. Male Sprague-Dawley rats, weighing 325 to 350 g at the beginning of drug treatment, were housed for ≥1 week before experimental manipulation in groups of two or three in wire-mesh cages with *ad libitum* access to food and water in a temperature- and humidity-controlled room and maintained on a 14-hr light (5:00 a.m. to 7:00 p.m.)/10-hr dark cycle. Animals were obtained from Simonsen Labs (Gilroy, CA).

Apparatus. Behavior was monitored in custom-designed activity chambers (Segal and Kuczenski, 1987). Briefly, each of the chambers was located in a sound-attenuated cabinet maintained on a 14-hr/10-hr light-dark cycle with constant temperature (20°C) and humidity (55 ± 5%). Each chamber consisted of two compartments: an activity/exploratory compartment (30 × 20 × 38 cm) and a smaller "home" compartment (14 × 14 × 10 cm) in which food and water were available *ad libitum*. Movements of the animal between quadrants within the activity/exploratory compartment (crossovers) and rearings against the wall, as well as eating and drinking and other vertical (*e.g.,* contact with a hanging stimulus) and horizontal movements (*e.g,.* intercompartment crossings) were monitored continuously by computer. Except where otherwise noted, crossovers and intercompartment crossings exhibited similar patterns. Therefore, these measures were combined to provide an index of all horizontal movement and are presented in the figures as "Crossings." In our previous binge/run treatment studies, observations of animals during the locomotor phase suggested that the enhanced locomotion was typically displayed in the form of burst-like patterns, that is, during these burst episodes, most crossovers between quadrants were made in rapid succession of one another (*i.e.,* 0–2 sec). To obtain a quantifiable index of this increased rate of movement, we added to our data collection system the capability of separately monitoring the crossovers made within a 0- to 2-sec ICI (*i.e.,* 0–2 sec ICI). We found that when this measure is presented as a percentage of total crossovers, it provides an accurate index of the occurrence of the burst patterns. In particular, we have found that values between 50% and 70% for this measure are obtained when the burst pattern is clearly observable. At lower values (*i.e.,* <35%), activity appears to be more uniform and recurrent bursts of locomotion are not detectable. Therefore, this measure represents a quantitative index that in conjunction with our observational ratings (now extended to include the poststereotypy locomotor period of the response) provides an accurate reflection of the changes in the pattern of locomotion that emerge with repeated run exposures.

In addition to the computer-monitored behaviors, representative animals were simultaneously videotaped for 60 sec at successive 5-min intervals for ≤8 hr to assess the qualitative features of the response during both the stereotypy and poststereotypy phases. Raters who were unaware of the specific experimental conditions subsequently rated the videotapes on the basis of behavior ethograms and rating procedures established previously (Segal and Kuczenski, 1987). Stereotypy was assessed as the percentage of the observation interval during which the animal displayed each specific behavior. The appearance of other atypical responses or behavior patterns, undetectable by our automated methods, were noted by the rater after each sampling interval. Because of the magnitude of the experiment, it was not possible to videotape sample behaviors from all animals; therefore, sets of rats (n = 5–7) from the most relevant groups were randomly selected for observational ratings.

Drugs. Amphetamine sulfate (NIDA, Rockville, MD) and methamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) were dissolved in saline and administered subcutaneously (at 2 ml/kg to avoid local irritation, which might be produced by high concentrations). Doses represent the free base. For comparative purposes, equimolar doses of AMPH and METH were selected.

General procedures. Three days before the beginning of drug treatment, animals were placed in individual experimental chambers, where they remained for the duration of the experiment. For all experiments, there were 9 to 11 rats in each group. To facilitate habituation to the chambers and procedures, animals were handled and injected with saline at least once a day. During the remainder of the day and night, animals were not disturbed, and their behavior was continuously monitored. Preliminary studies revealed no significant differences in the responses to acute drug administration of

cross-sectional control groups, indicating this 3-day period to be sufficient habituation for the prolonged residence in the chambers.

After the habituation period, groups of animals received 15 SDI of 2.5 or 4.0 mg/kg AMPH or 4.42 mg/kg METH (the METH dose selected is equimolar to 4.0 mg/kg AMPH). Drug was administered in cycles of 5 successive days, followed by 2 days of saline injections. Control groups received an equivalent number of saline injections. At 24 hr after the 15th drug injection (*i.e.,* after the third drug cycle), the experimental and control groups received a challenge injection of the same doses of drug (day 20). Groups that continued on to the binge phase of this experiment received one injection of saline on day 21 and binge injections were initiated on day 22. In our earlier studies (Segal and Kuczenski, 1997), because of the high-dose of AMPH used in the run (8 mg/kg), we used an escalating dose pretreatment phase to enable tolerance to develop to the physiological and behavioral toxicity (*e.g.,* convulsions, ataxia, persistent high-core temperature or lack of grooming) associated with these high doses. In the present study, we administered lower doses during binges, and pilot studies revealed that after pretreatment with as few as 6 SDI of the same dose, there was no evidence of behavioral toxicity in response to subsequent run exposures. During the run phase, animals received the same dose every 2 hr, for four injections, beginning at 8:00 a.m. and ending at 2:00 p.m. Animals were exposed to this daily binge regimen for three cycles, with each cycle consisting of 5 consecutive binge days, followed by 2 days of saline. Two days after the third binge cycle, animals were challenged with a single injection of the same dose of drug.

For dialysis studies, animals were stereotaxically implanted with guide cannulae using procedures previously described in detail (Kuczenski and Segal, 1989). Guide cannulae extended 2.6 mm below the surface of the skull and were aimed at the caudate-putamen (1.0 mm anterior to bregma, 2.8 mm lateral and 6.2 mm below dura) or the nucleus accumbens (2.2 mm anterior, 1.5 mm lateral and 7.8 mm below dura). After surgery, animals were housed individually and allowed $\geq$1 week to recover before receiving any treatment.

Groups of animals were pretreated with 6 SDI of 4.0 mg/kg AMPH or 4.42 mg/kg METH, and at 48 hr after the last injection were exposed to a first binge with the same drug and dose. Pilot studies indicated that the behavioral responses to a first binge after 6 or 15 SDI were essentially identical. On the day before the binge (3:00–4:00 p.m.), each rat was placed in an experimental chamber, and the dialysis probes were inserted to allow for acclimation to the test environment and adequate equilibration of the dialysis probes. The dialysis chambers were essentially identical to the behavioral chambers described above, with the exceptions that the "home" compartment and hanging stimulus were removed to prevent interferences introduced by the dialysis methodology. Concentric microdialysis probes were constructed of Spectra/Por hollow fiber (MW cutoff 6000, o.d. 250 μm) as previously described (Kuczenski and Segal, 1989). The length of the active probe membrane was 3 mm for caudate-putamen and 1.5 mm for nucleus accumbens. Probes were perfused with artificial cerebrospinal fluid (147 mM NaCl, 1.2 mM $CaCl_2$, 0.9 mM $MgCl_2$, 4.0 mM KCl) delivered by a microinfusion pump (1.5 μl/min) *via* 50 cm of Micro-Line ethyl vinyl acetate tubing connected to a fluid swivel. Dialysate was collected through glass capillary tubing into vials containing 20 μl of 25% methanol and 0.2 M sodium citrate, pH 3.8. Under these conditions, dialysate DA and 5-HT and metabolites were stable throughout the collection and analysis interval. Samples were collected outside the experimental chamber to avoid disturbing the animal. Individual probe recoveries were estimated by sampling a standard DA solution *in vitro*. Preliminary studies indicated that individual probe recoveries for DA and 5-HT were similar. At the end of the experiment, each animal was perfused with formalin for histological verification of probe placements.

Dialysate samples were collected every 30 min and were assayed for DA, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-methoxytyramine, 5-hydroxyindoleacetic acid and 5-HT. In all experiments, solutions of standards revealed a clean separation between 3-methoxytyramine and 5-HT. The HPLC-EC consisted of a 100 $\times$ 4.6 mm ODS-C_{18} 3-μm column (Regis) maintained at 40°C. Mobile phase (0.05 M citric acid, 7% methanol, 0.1 mM Na_2EDTA and 0.2 mM octane sulfonate adjusted to pH 4.0–4.5) was delivered at 0.6 to 0.8 ml/min by a Waters model 510 pump. Amines were detected with a Waters 460 detector with a glassy carbon electrode maintained at +0.65 V relative to a Ag/AgCl reference electrode. Concentrations were estimated from peak areas using a Waters Maxima 820 data station. Substances in the dialysates were corrected for individual probe recoveries to account for this source of variability, and although the exact relationship between dialysate concentration and actual extracellular transmitter content is not clear (Benveniste *et al.*, 1989; Church *et al.*, 1987; Stahle *et al.*, 1991; Wages *et al.*, 1986), values are presented as dialysate concentration to allow meaningful comparisons to other data in the literature.

Analyses of AMPH and METH in brain tissue were performed on the trifluoroacetic anhydride derivatives using a deuterated internal standard (AMPH-d3) on a HP5971A GC/MS system with fused silica capillary column coated with methylsilicone as previously described (Melega *et al.*, 1995).

Data analysis. Behavioral and neurochemical data were statistically analyzed using repeated-measures ANOVA and *t* tests with Bonferroni's corrections for specific group/time comparisons.

Results

Behavior. With repeated SDI of the equimolar doses of AMPH (4.0 mg/kg) and METH (4.42 mg/kg), both drugs exhibited a progressive pattern of response alteration (figs. 1-3) typical of this dose range (for recent reviews, AMPH: Rebec and Segal, 1980; Segal, 1975; METH: Machiyama, 1992; Sato *et al.*, 1992), and by the 16th injection, there were no significant differences apparent between the two drugs with respect to the magnitude or temporal features of the two primary behavioral components of the response.

The response to SDI of 4.0 mg/kg AMPH stabilized by about the fifth injection so that further intermittent drug administration did not significantly affect either the stereotypy or locomotor components of the response (fig. 1). As frequently occurs with SDI of moderate to high-doses of AMPH, the poststereotypy locomotion phase was not prolonged, and in fact slightly decreased (Segal, 1975; Segal *et al.*, 1995; Segal and Kuczenski, 1997). Therefore, only the first half of this period (90–180 min) exhibited significant sensitization by the fifth injection (day 1 *vs.* day 5: 145 $\pm$ 19 *vs.* 288 $\pm$ 37, t = 4.44, P $<$ .01). In contrast, subsequent repeated exposure to daily runs resulted in a relatively selective and pronounced increase in the magnitude of poststereotypy locomotor activation, as evident in the locomotor response pattern to the fourth injection of the 15th run (fig. 1). (Except where otherwise noted, references to characteristics of the run response refer to the fourth injection.) The duration of the continuous stereotypy phase did not correspondingly increase with successive runs and, in fact, was slightly shorter (fig. 3, top). Furthermore, although the intensity of stereotypy during the first run significantly increased compared with the last SDI (*i.e.,* mild licking and biting became a moderate-to-strong biting response; data not shown), the characteristics of the stereotypy did not change further during subsequent runs.

The videotaped observations of the animals did reveal a profound change in the qualitative features of the poststereotypy locomotion. As previously noted for higher dose runs

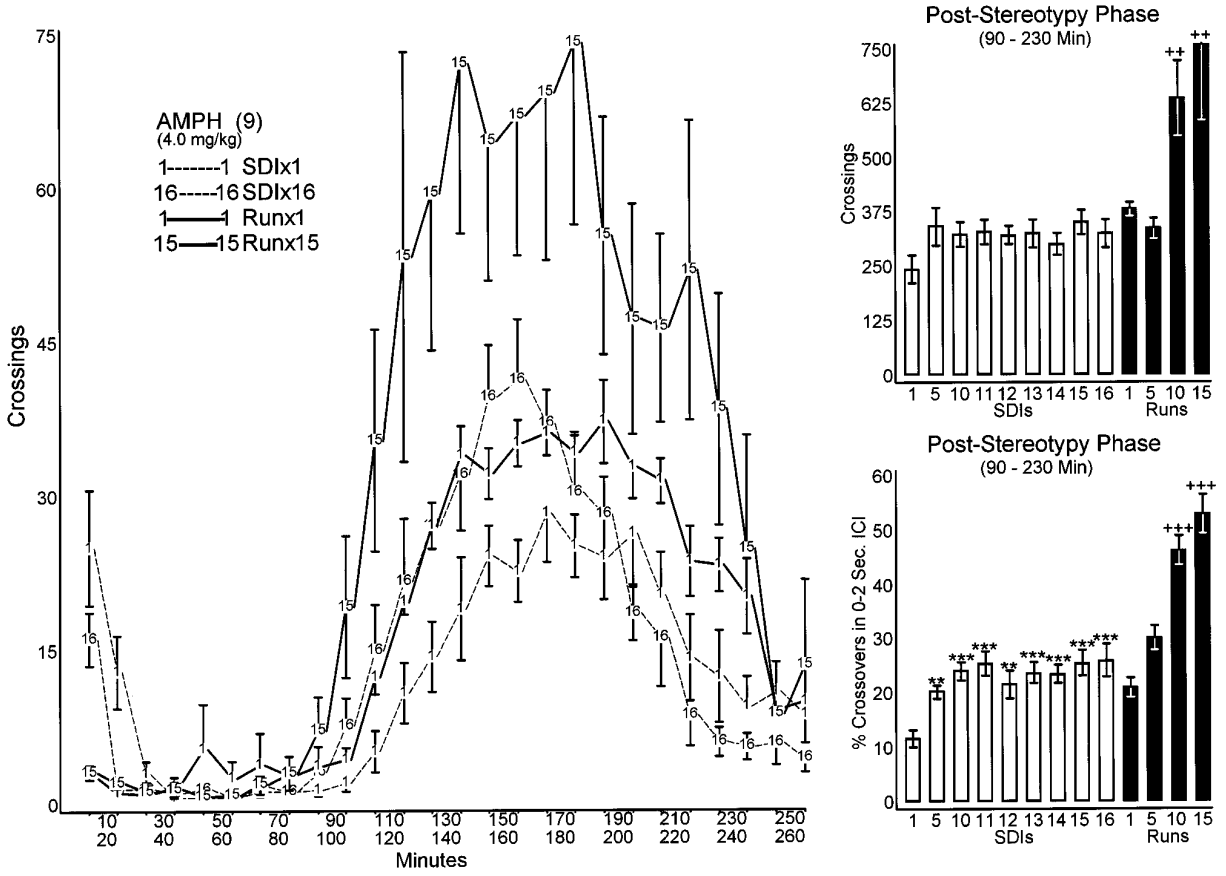

Fig. 1. Horizontal locomotion in response to the first and 16th SDI of AMPH (4.0 mg/kg) and the fourth injection of the first and 15th AMPH run. Histograms represent cumulated crossings (top) or percentage of total crossovers occurring within the 0- to 2-sec ICI (bottom) during the indicated interval in response to representative SDIs and the fourth injection of subsequent runs (crossings: ANOVA, effect of runs, $F = 6.3$, $P < .001$, all $t > 3.3$; fast crossovers: ANOVA, effect of SDIs, $F = 9.0$, $P < .001$, all $t > 3.4$; ANOVA, effect of runs, $F = 28.5$, $P < .001$, all $t > 5.3$). **, $P < .01$, ***, $P < .001$ compared with the response to the first SDI. ++, $P < .01$, +++, $P < .001$ compared with the response during the 16th SDI.

(Segal and Kuczenski, 1997), during this phase of the response, rats exhibited an "agitated" behavioral profile, characterized by episodes of burst-like locomotion during which crossovers were made within rapid succession of each other (*i.e.,* <2-sec ICI). Examination of crossovers during this 0- to 2-sec ICI as a percentage of total crossovers revealed that during the course of SDI, the proportion of rapid rate crossovers significantly increased from ~10% to 12% during the acute response to ~20% to 25% after about five daily injections (fig. 1). Observations indicated that this change reflected a relatively uniform increase in the rate of movement (*i.e.,* there were no episodic bursts of activity). No further significant change in rapid rate crossovers occurred during the remainder of the SDI or during the initial exposure to runs. However, by run 15, >50% of the crossover activity occurred within the 0- to 2-sec ICI (fig. 1), and this activity was exhibited primarily in the form of discrete bursts. Furthermore, unlike the response to SDI, during which animals often paused between crossovers or engaged in behaviors such as grooming or interaction with the hanging stimulus, the behavior between run-induced bursting appeared to be more intense and considerably less varied (*i.e.,* primarily nose-poking).

A similar pattern of behavioral change resulted from multiple run exposures to the equimolar dose of METH (4.42 mg/kg). The poststereotypy phase was characterized by both the enhanced magnitude and the predominance of burst-like locomotion (fig. 2) as well as by the decrease in the duration of the continuous stereotypy phase relative to the acute response (fig. 3, bottom). One pronounced difference, however, was that although the magnitude of the poststereotypy response was comparable for the two drugs, the duration of the METH response to the fourth injection of the run was markedly prolonged by ~2 hr (crossings, 320–380-min interval; 4.0 mg/kg AMPH: 3.4 ± 1.9; 4.42 mg/kg METH: 109.3 ± 22.6; $t = 4.66$, $P < .001$). This difference was apparent during the first run and persisted through the course of subsequent run exposures. Importantly, however, there was no corresponding increase in the duration of the stereotypy phase during the METH runs (fig. 3).

The unique run-induced behavioral profile persisted in response to a challenge injection of each drug for ≥3 days after the last run exposure. Thus, the poststereotypy response to a challenge injection was substantially augmented compared with the response to the 16th SDI, for both AMPH (fig. 4, top) and METH (fig. 4, bottom). Likewise, qualitative differences in the poststereotypy locomotion, including the burst pattern of activity, also distinguished these responses from the effect produced by SDI. In addition, although the challenge response to AMPH was not significantly longer than during SDI, a significant prolongation was detected for METH (250–280 min: $F = 4.75$; $P < .01$). In contrast, the

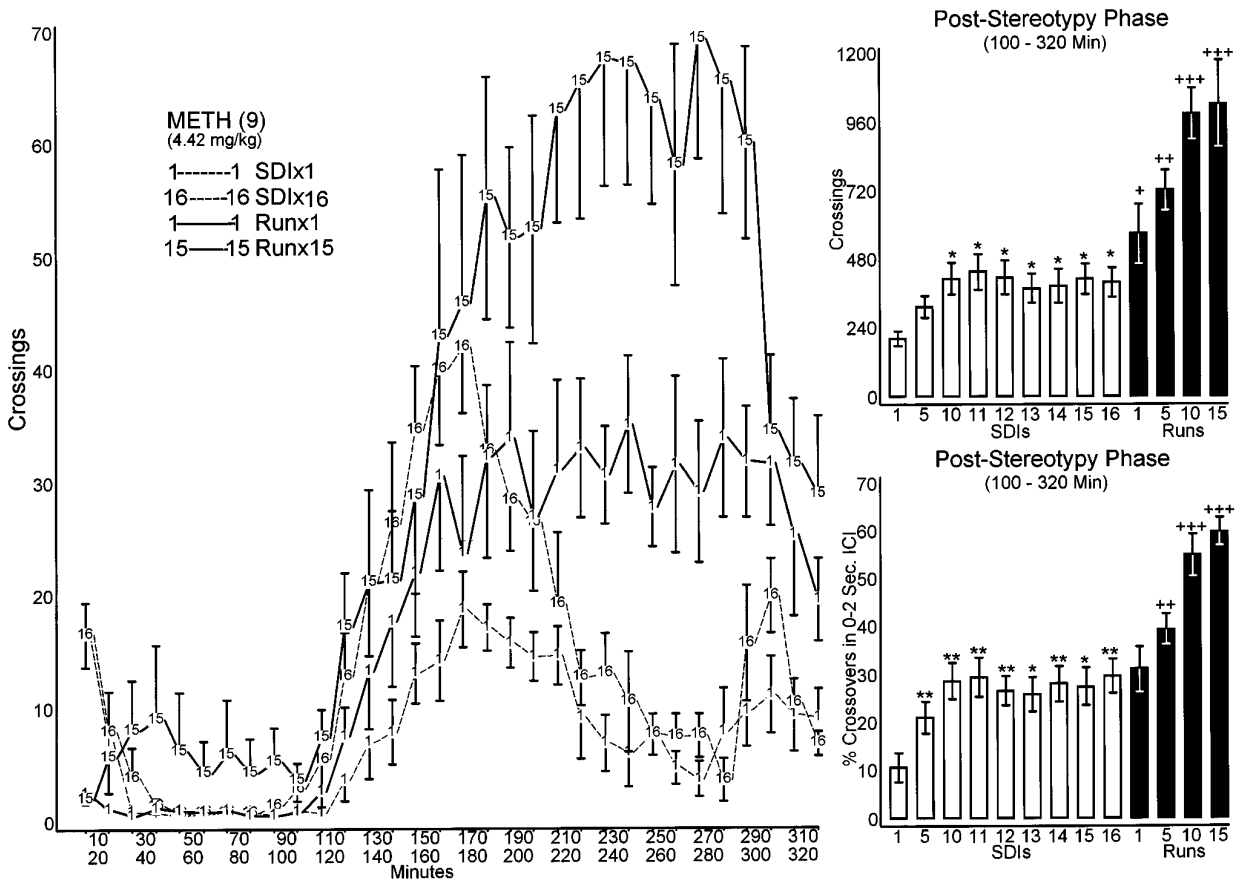

Fig. 2. Horizontal locomotion in response to the first and 16th SDI of METH (4.42 mg/kg) and the fourth injection of the first and 15th METH run. Histograms represent cumulated crossings (top) or percentage of total crossovers occurring within the 0- to 2-sec ICI (bottom) during the indicated interval in response to representative SDIs or the fourth injection of subsequent runs (crossings: ANOVA, effect of SDIs, $F = 6.1$, $P < .001$, all $t > 2.5$; ANOVA, effect of runs, $F = 9.4$, $P < .001$, all $t > 2.7$; fast crossovers: ANOVA, effect of SDIs, $F = 6.9$, $P < .001$, all $t > 2.9$; ANOVA, effect of runs, $F = 45.1$, $P < .001$, all $t > 9.0$). *, $P < .05$, **, $P < .01$ compared with the response to the first SDI. +, $P < .05$, ++, $P < .01$, +++, $P < .001$ compared with the response during the 16th SDI.

stereotypy responses to the 16th SDI and the challenge injection in run-exposed animals were comparable in duration for both drugs (fig. 4).

Virtually all these behavioral profile changes that were observed to occur with the higher doses of AMPH and METH were also found in response to the lowest dose of AMPH (*i.e.,* 2.5 mg/kg) that, under our experimental conditions, reproducibly elicits a multiphasic response pattern. The changes in the response with successive run exposures to 2.5 mg/kg AMPH were similar to the emergent behavioral pattern associated with the higher doses. By the fourth injection of each run (fig. 5), the only change in the temporal pattern of the continuous stereotypy phase was a more rapid onset of stereotypy compared with the 16th SDI (0–10 min: SDI × 16 *vs.* run × 1 and run × 15, 30 ± 12 *vs.* 100 ± 0 and 92 ± 8; ANOVA for treatments, $F = 12.6$, $P < .001$; all $t > 5.8$, all $P < .01$). By contrast to the minimal changes in the magnitude and duration of the continuous stereotypy phase, the poststereotypy locomotion was significantly enhanced by the 15th run (fig. 5), and, as with the higher dose runs, the rapid movement crossovers (within the 0–2-sec ICI) also increased with multiple runs. The acute response crossovers during the 0- to 2-sec ICI were ~10% to 15% of total crossovers; this value significantly increased to 25% to 30% ($t = 3.4$, $P < .01$) between the fifth and 10th SDI and then remained stable during subsequent SDI. However, after multiple runs, the ICI measure substantially increased, achieving a level of ~50% by the 15th run. Observations of these animals revealed the emergence of a bursting pattern of activity corresponding to the increase in the intercrossover rate measure. Preliminary results indicated a similar pattern of behavioral change with multiple run exposures to an equimolar dose of METH (2.76 mg/kg).

Challenge with AMPH (2.5 mg/kg) 3 days after the 15th run exposure revealed the persistence of the unique run-induced behavioral pattern (fig. 6). These results paralleled the effects of the higher doses of both drugs with respect to both the stereotypy and locomotor components of the response. That is, in contrast to the trend toward a diminished continuous stereotypy phase, the poststereotypy locomotion remained significantly enhanced and was characterized by a burst pattern of activity.

Neurochemistry. We had previously shown that high-dose (8.0 mg/kg AMPH) multiple run exposure resulted in a depletion of caudate-putamen DA of ~25% (Segal and Kuczenski, 1997). To assess further the possible role of DA depletion in the run-induced behavioral response, we examined caudate-putamen DA 3 days after 12 daily AMPH and METH runs. Both higher-dose groups exhibited significant DA depletions of ~15% [control, 108 ± 4 pmol/g; 4.0 mg/kg AMPH,

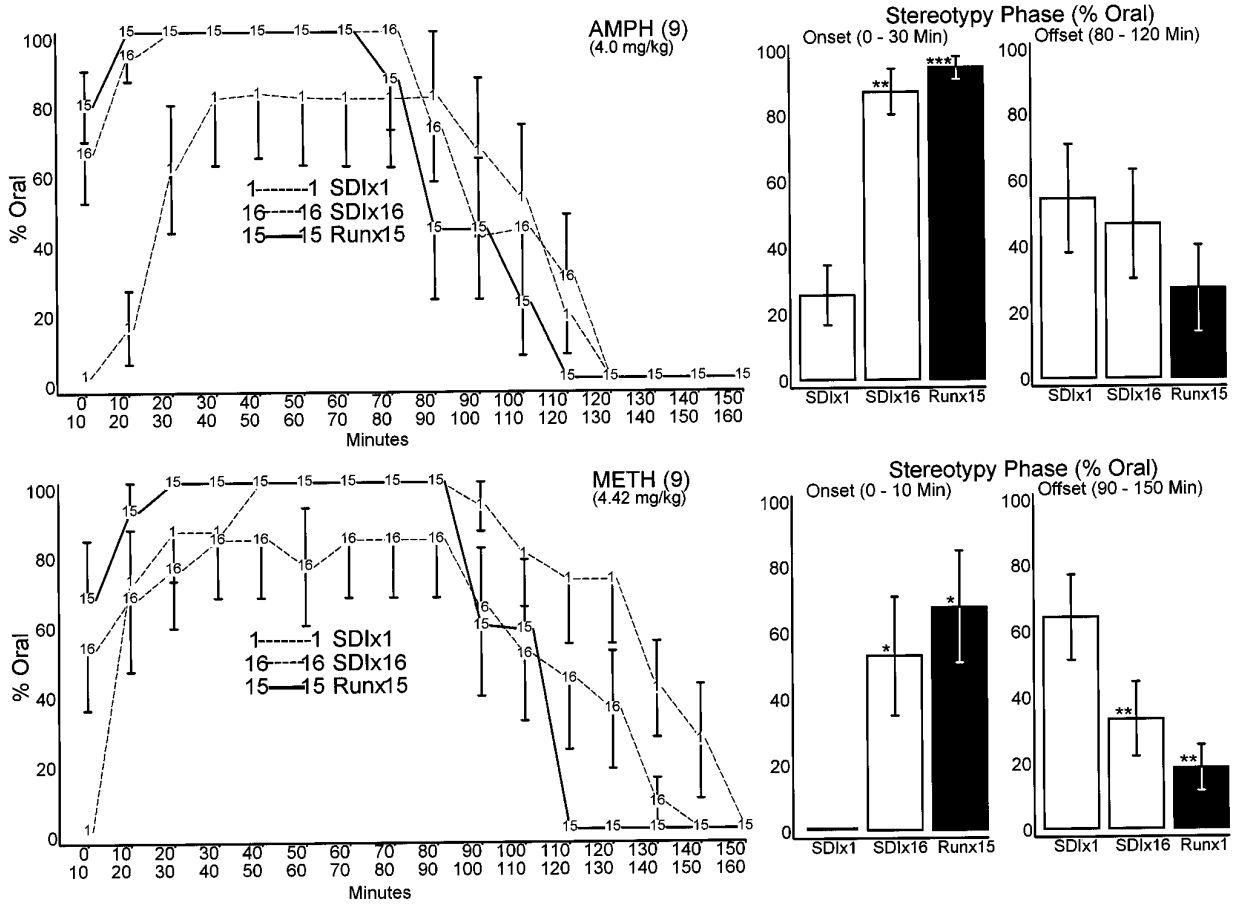

Fig. 3. Temporal pattern of the oral stereotypy response to the first and 16th SDI and the fourth injection of the 15th run of AMPH (4.0 mg/kg) (top) or an equimolar dose of METH (4.42 mg/kg) (bottom). Histograms represent the cumulated response over the indicated interval (AMPH onset: ANOVA, $F = 28.7$, $P < .001$, all $t > 4.9$; METH onset: ANOVA, $F = 7.2$, $P < .02$, all $t > 2.9$; METH offset: ANOVA, $F = 9.3$, $P < .01$, all $t > 3.6$). *, $P < .05$, **, $P < .01$, ***, $P < .001$ compared with the response to the first SDI.

86 ± 4 pmol/g ($t = 3.73$, $P < .01$); 4.42 mg/kg METH, 84 ± 4 pmol/g ($t = 4.08$, $P < .01$), whereas neither lower-dose group exhibited a significant change in DA levels (2.5 mg/kg AMPH, 97 ± 4 pmol/g; 2.76 METH, 100 ± 7 pmol/g).

The caudate-putamen and nucleus accumbens extracellular DA and 5-HT responses to run exposure with AMPH (4.0 mg/kg) or METH (4.42 mg/kg) were examined to assess the response profile associated with run pattern of stimulant administration. Microdialysis was performed during a first run in groups of animals pretreated with 6 SDI of 4.0 mg/kg AMPH or 4.42 mg/kg METH, and the results are summarized in figures 7 and 8. The caudate-putamen and nucleus accumbens exhibited similar DA profiles in their relative responses to the two drugs (fig. 7). In caudate-putamen, the METH response tended to be higher than the AMPH response ($P = .06$) when AUC was compared during the successive 2-hr intervals after each injection and was significantly higher for the second hour after each injection ($F = 6.49$, $P < .02$) and for the 600- to 720-min interval (the last 2 hr we measured) of the overall response ($t = 2.54$, $P < .02$). In nucleus accumbens, METH promoted a significantly greater DA response when AUC was compared over the successive 2-hr intervals after each injection ($F = 10.61$, $P < .001$), as well as during the last 2 hr of the overall response ($t = 4.51$, $P < .01$). Both groups produced a significant decrease in the caudate-putamen DA response (AUC) as a function of successive injections within the run (all $F > 2.91$, all $P < .05$), whereas neither group exhibited a significant injection effect for nucleus accumbens DA.

The 5-HT response was significantly higher to METH compared with AMPH in nucleus accumbens (peak responses to first injection: 18.6 ± 2.2 *vs.* 7.8 ± 0.6 pmol/g; $t = 4.71$, $P < .001$) and tended to be higher in caudate-putamen. It should be noted that the caudate-putamen 5-HT response to AMPH (fig. 8, top) includes two animals with unusually high responses to the first injection. With exclusion of those animals, the 5-HT responses to METH would be significantly higher than AMPH). In contrast to the DA profile, for 5-HT (fig. 8), AMPH did not produce a significant injection effect in either caudate-putamen (with or without the unusually high 5-HT responders) ($P = .88$) or nucleus accumbens ($P = .25$), whereas METH-treated animals exhibited a significant injection effect in both caudate-putamen ($F = 14.8$. $P < .01$) and nucleus accumbens ($F = 3.31$, $P < .05$)

Because the run-induced prolongation of the locomotor and regional DA responses in the METH treated animals might be due to pharmacokinetic factors, we examined regional tissue levels of METH and its metabolite, AMPH, at a time during the run (3.5 hr after the fourth injection of the first or sixth 4.42 mg/kg METH run) when animals exhibited a pronounced increase in locomotor activation relative to the acute and the SDI responses. (crossings, 180–240-min interval;

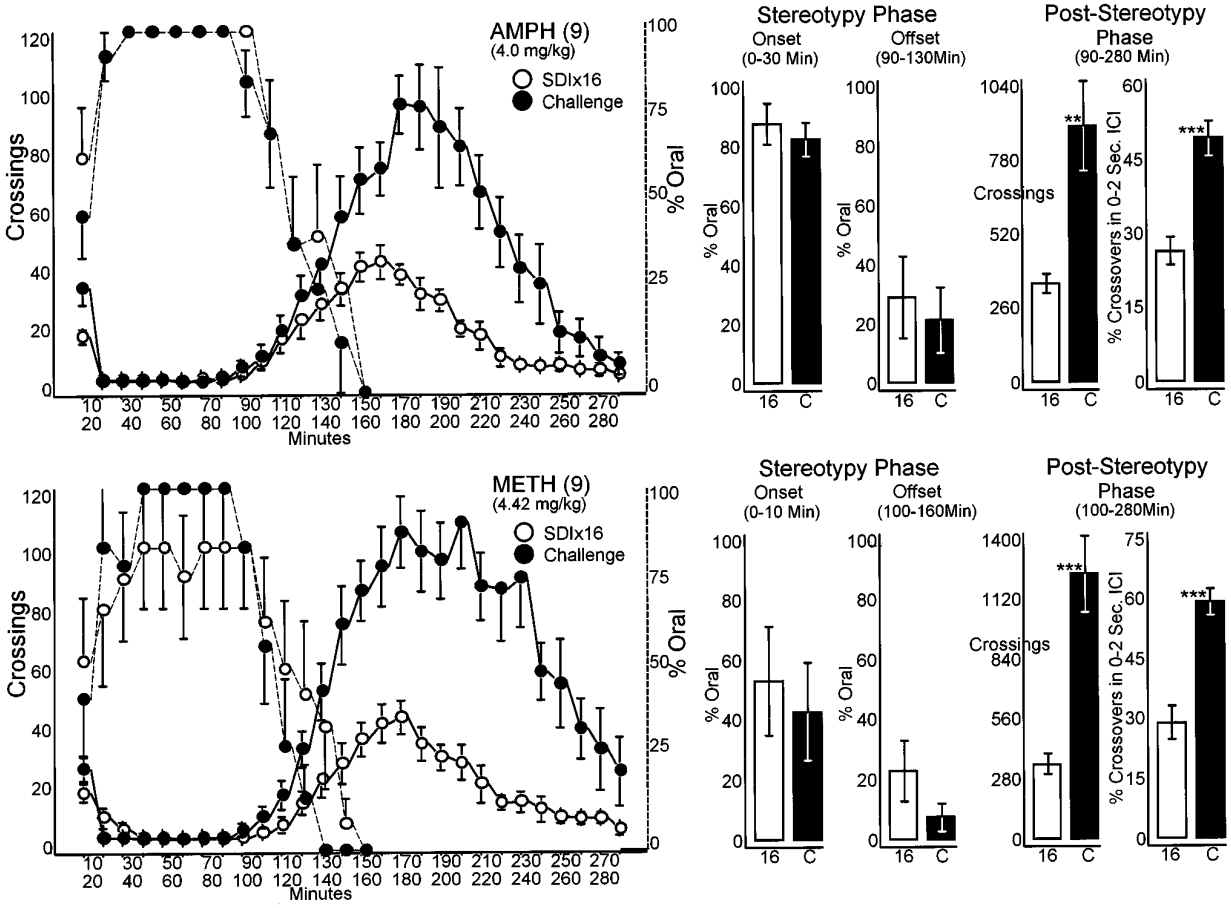

Fig. 4. Effects of exposure to multiple runs on the temporal patterns of horizontal locomotion (solid lines) and oral stereotypy (dashed lines) in response to challenge with AMPH (4.0 mg/kg) (top) and METH (4.42 mg/kg) (bottom) 3 days after the last of 15 daily runs. The challenge response is compared with the corresponding 16th SDI. Stereotypy is presented as percentage of the interval during which animals were engaged in oral (continuous licking or biting) behaviors. Numbers in parentheses represent number of animals. Histograms depict the cumulated response (oral stereotypies, crossings or percentage of total crossovers occurring within the 0–2-sec ICI) over the indicated interval. All t values >3.4; **, $P < .01$, ***, $P < .001$ comparing the response after the challenge injection to the response of the 16th SDI.

acute: 84.7 ± 18.0; SDI × 6: 87.3 ± 14.5; run 1: 321.3 ± 36.7; run 6: 566.0 ± 52.3; ANOVA, effect of treatment: $F = 68.4$, $P < .001$; all $t > 5.6$, all $P < .001$). At this time point during the first run, METH and its AMPH metabolite were significantly greater in caudate-putamen (~2-fold) than levels in acute or SDI-treated controls (table 1). In addition, the AMPH metabolite was further increased in cortex compared with caudate-putamen. With additional runs, no further increases in drug or metabolite levels were observed.

Discussion

We previously found (Segal and Kuczenski, 1997) that a unique behavioral and neurochemical profile emerged in response to a stimulant administration regimen that attempted to simulate the drug abuse regimen most commonly associated with addiction and the induction of various forms of psychopathology. The results of the present series of studies support and extend these observations to repeated drug-on/drug-off exposure cycles of lower doses of both AMPH and METH and further characterize the behavioral and neurochemical changes associated with this treatment exposure.

During the repeated SDI phase preceding the runs, the response to both AMPH and METH exhibited patterns of change characteristic of the respective doses, including elements of sensitization as previously described (see Results and above for references). The locomotor patterns did not display further significant change after ~5 to 10 SDI. These results, along with our previous findings (Segal and Kuczenski, 1997), indicate that with SDI of doses ranging from 2.5 to 8.0 mg/kg, response alterations develop relatively fast and then remain stable with further SDI.

However, during subsequent exposure to runs, further alterations in the response developed, with some changes apparent during the first run. For both doses of AMPH, although the temporal pattern of the locomotor response did not change from the last SDI, the stereotypy increased in intensity and exhibited a more rapid onset for the lower dose (2.5 mg/kg). The response to the first run of METH was similar to the equimolar dose of AMPH, except that the poststereotypy locomotor activity was markedly prolonged. This occurred without any corresponding increase in the duration of the stereotypy phase, as would be expected in response to increasing doses. The difference in response duration between AMPH and METH persisted throughout the run phase of the study. As with AMPH, however, the qualitative characteristics of the locomotor response during the first METH run did not differ significantly from its features during SDI.

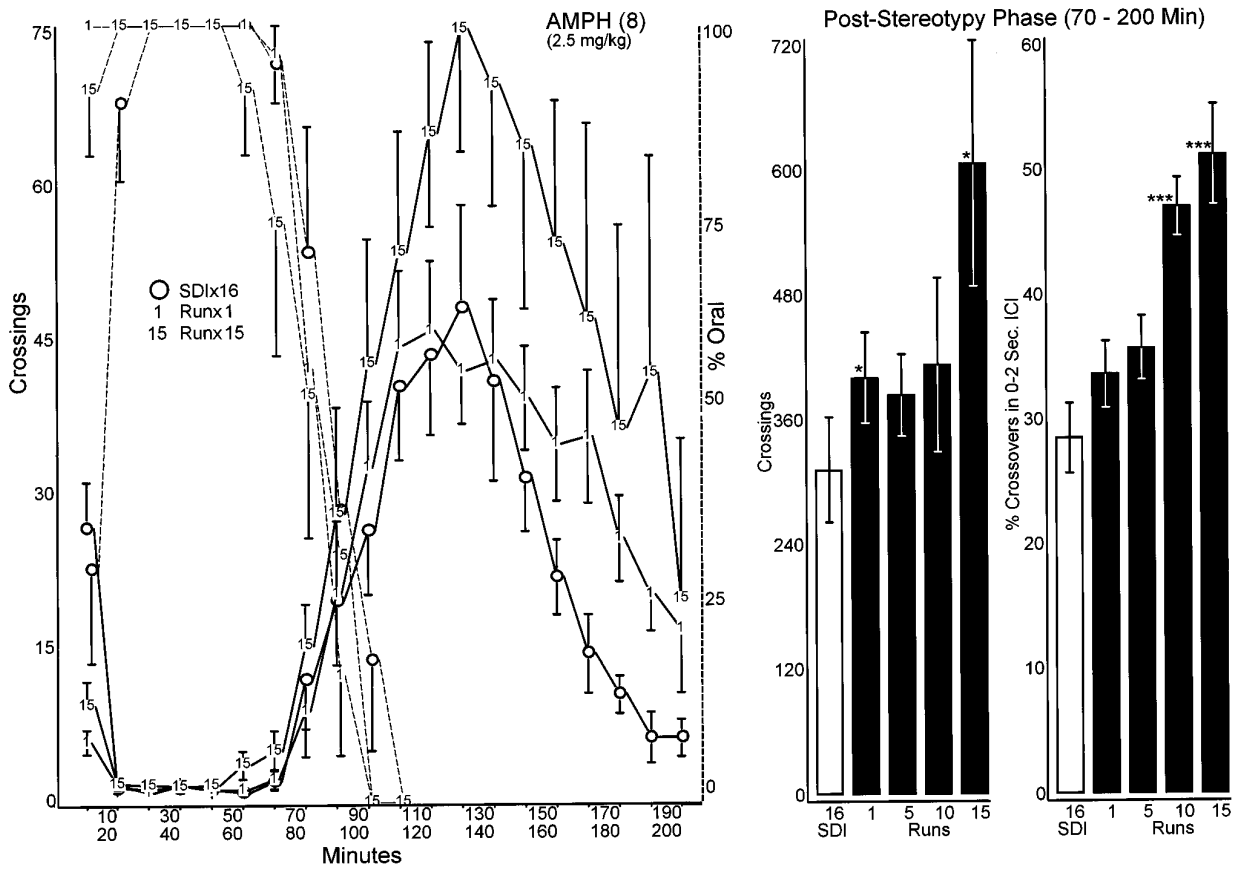

Fig. 5. Temporal patterns of the locomotor (solid lines) and stereotypy (dashed lines) responses to the 16th SDI compared with the fourth injection of the first and 15th run with AMPH (2.5 mg/kg). Stereotypy is presented as percent of the interval during which animals were engaged in oral (continuous licking or biting) behaviors. Histograms depict the cumulated response (crossings and percentage of total crossovers occurring within the 0–2-sec ICI) over the indicated interval (crossings: ANOVA, $F = 3.5$, $P < .05$, all $t > 3.1$; fast crossovers: ANOVA, $F = 14.2$, $P < .001$, all $t > 6.0$). *, $P < .05$, ***, $P < .001$ compared with the corresponding response to the 16th SDI.

For both drugs, with multiple runs, the magnitude and qualitative characteristics of the poststereotypy locomotor activation were significantly altered. In contrast, most features of the continuous stereotypy phase either did not change or exhibited tolerance. Although the rate of development of this unique behavioral profile appeared to be dose dependent, the response ultimately achieved characteristics similar to the profile we previously observed with a considerably higher dose of AMPH (Segal and Kuczenski, 1997). With successive runs, the most prominent change in the poststereotypy locomotion was an increase in the magnitude of the locomotor response. In the present study, observational ratings that were now extended beyond the continuous stereotypy phase permitted a more complete characterization of this behavior. These observations indicated that there was a corresponding change in the qualitative features of the enhanced locomotor response. Rather than the more uniform expression of crossings associated with acute and single daily injections, after multiple runs most of the locomotion occurred in the form of episodic bursts of activity. Furthermore, although the locomotor response to SDI often included rearing, grooming and even pauses, rats that received successive run exposures appeared to be in a continuous state of extreme agitation (*i.e.,* even when not ambulating, their heads and/or limbs were constantly moving). Between bursts, most animals, irrespective of dose or drug, appeared to engage primarily in nose-poking behavior, although at the higher doses, occasional biting and licking were apparent, especially during the transition between the stereotypy and locomotor phases of the response.

The burst-like behavior was also reflected quantitatively, in the increased proportion of rapid rate crossovers (*i.e.,* crossovers within the 0–2-sec ICI). Our results indicated that within the 2.5 to 8.0 mg/kg AMPH dose range, only the pattern of drug administration altered this rate measure. That is, after acute drug injection, the proportion of crossovers within the 0- to 2-sec ICI was typically 10% to 15% of total crossovers, independent of dose and, therefore, of the total activity during the poststereotypy period. This rate measure increased to ~25% to 30% after SDI, with dose only influencing the number of injections required to achieve this level. During the first several run exposures, there were no further changes in the rate measures. However, corresponding to the appearance of bursting (between 5 and 15 runs, depending on dose), rapid rate crossovers increased to levels of ~50% to 60% of total crossovers for all doses of both AMPH and METH tested. These results are consistent with observations that after multiple runs, most of the locomotor activity occurred in a bursting pattern, with varying intervals between successive bursts. This behavioral change was apparent throughout the poststereotypy locomotor period produced by both AMPH and METH runs, indicating that the prolongation of the METH response likely involves mecha-

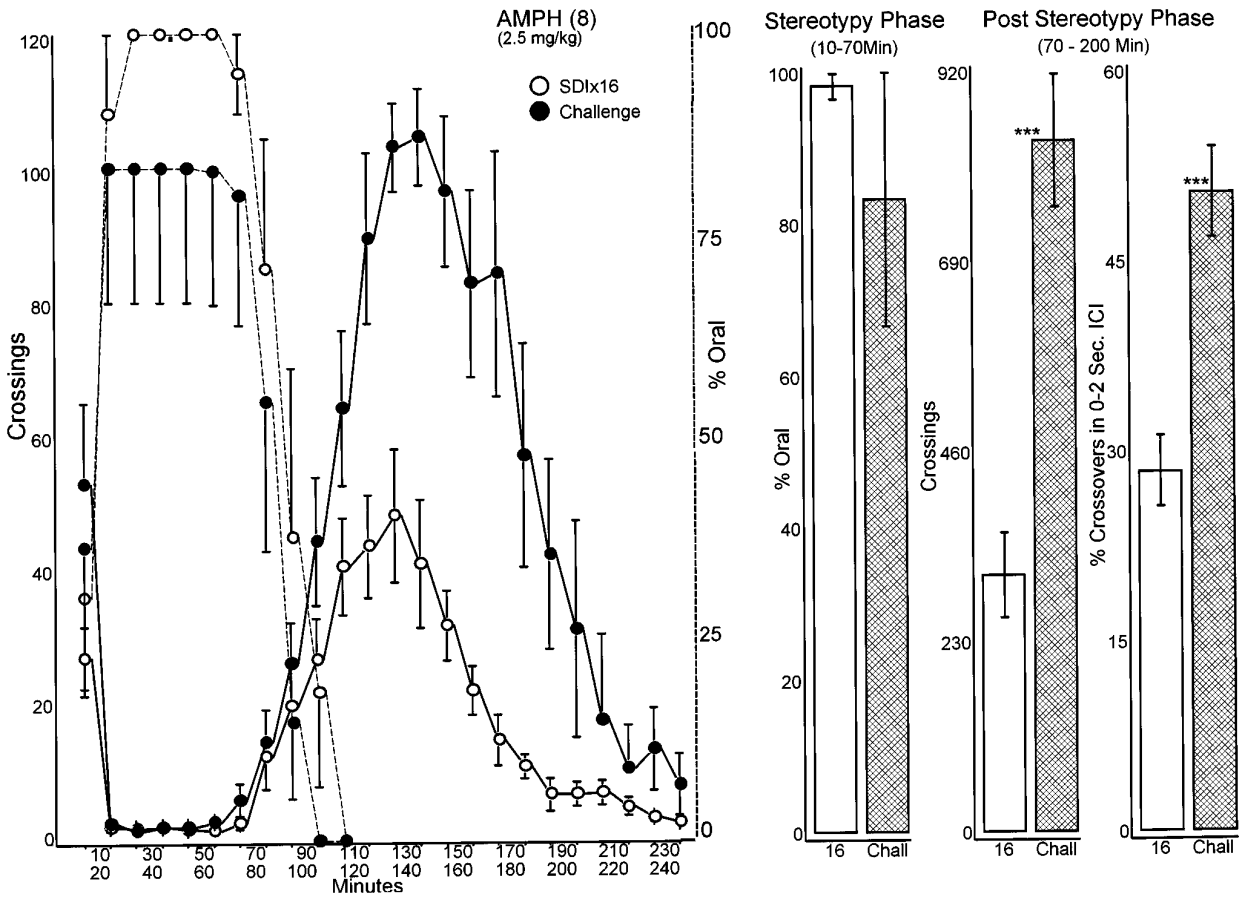

Fig. 6. Effects of exposure to multiple runs on the temporal patterns of horizontal locomotion (solid lines) and oral stereotypy (dashed lines) in response to a challenge injection of AMPH (2.5 mg/kg) 3 days after the last of 15 daily runs. The challenge response is compared with the 16th SDI. Stereotypy is presented as percent of the interval during which animals were engaged in oral (continuous licking or biting) behaviors. Histograms depict the cumulated response (oral stereotypies, crossings or percentage of total crossovers occurring within the 0–2-sec ICI) over the indicated interval. All t values > 5.1; ***, $P < .001$ comparing the response after the challenge injection (Chall) to the response of the 16th SDI (16).

nisms different from those responsible for the burst pattern of locomotion (see below).

It is important to emphasize that this unique behavioral pattern requires multiple run exposures before it emerges; it does not occur in response to acute or intermittent administration of any dose tested (Segal, 1975; Segal and Kuczenski, 1997; present results), nor is it displayed during the initial runs. Therefore, cumulative drug dose achieved during the run is not a critical factor. Furthermore, although these changes result from frequent episodes of multiple daily drug injections, subsequent expression of the altered response does not require run exposure. This is most convincingly demonstrated in the response to challenge with an acute dose 3 days after the last run. Our previous findings indicated that these effects persisted for ≥3 weeks after the last 8.0 mg/kg AMPH run (Segal and Kuczenski, 1997). These observations suggest that the run-induced changes in behavior reflect a fundamental alteration in responsiveness to the drug.

The DA and 5-HT response profiles to successive injections of AMPH and METH exhibited transmitter- and region-specific differences. We previously reported that the caudate-putamen and nucleus accumbens DA responses within the first 8 mg/kg AMPH run declined to near 50% of the initial response (Segal and Kuczenski, 1997). A reduction in the caudate-putamen DA response also occurred during the first run with 4.0 mg/kg AMPH or 4.42 mg/kg METH, although the decline was substantially less (~20% of the initial peak response) than with the higher dose [peak DA (nM) first injection *vs.* fourth injection: AMPH, 290 ± 42 *vs.* 220 ± 30; $t = 2.67$, $P < .05$; METH, 361 ± 38 *vs.* 283 ± 29; $t = 3.03$, $P < .01$]. In contrast to the caudate-putamen, the nucleus accumbens DA responses exhibited no significant decrement. These results are consistent with a dose-dependent tolerance/tachyphylaxis in the DA response within runs and a region-dependent sensitivity to this treatment. In this regard, we previously suggested that a shift in the balance between mesostriatal *vs.* mesolimbic DA activation may contribute to the progressive change in the relationship between the expression of stereotypy and locomotion with multiple runs, and the present results are supportive of this hypothesis.

The progressive decline in caudate-putamen DA responsivity within a run may be related to the depletion of tissue levels of DA associated with multiple high-dose runs (Clausing *et al.*, 1995; Schmidt *et al.*, 1985a, 1985b; Segal and Kuczenski, 1997) because some evidence suggests that caudate-putamen DA is more sensitive to depletion than is nucleus accumbens (Castañeda *et al.*, 1990; Ellison *et al.*, 1978; Ellison and Eison, 1983; Paulson and Robinson, 1995). If the decline in responsivity reflects DA depletion, the absence of a

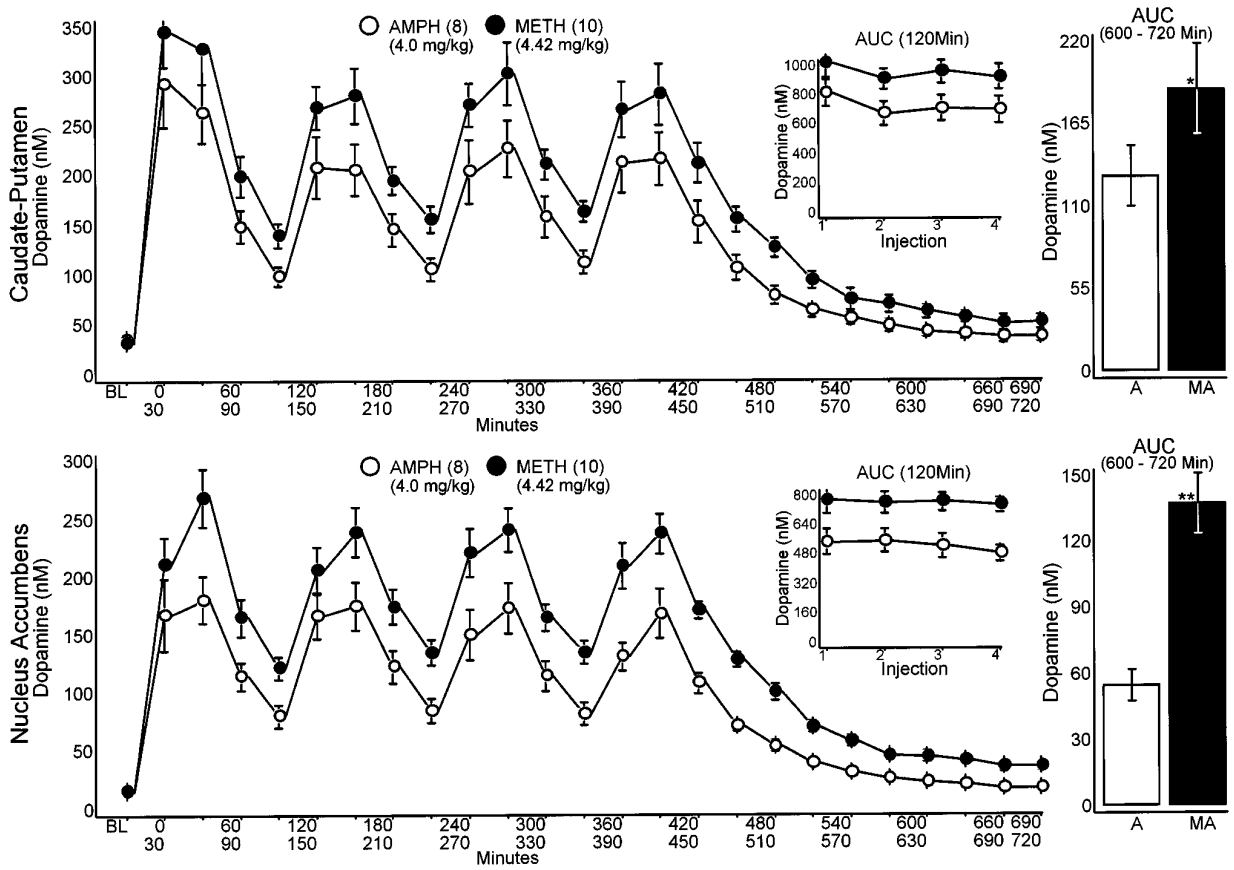

Fig. 7. Extracellular DA in caudate-putamen (top) and nucleus accumbens (bottom) during the first run of four successive injections at 2-hr intervals of AMPH (4.0 mg/kg) or METH (4.42 mg/kg). Insets, AUC of the DA response during the 120-min interval after each injection. ANOVA revealed a significant effect of injection for both AMPH ($F = 2.98$, $P < .05$) and METH ($F = 2.87$, $P < .05$) in caudate-putamen but not in nucleus accumbens. Histograms depict the AUC for the DA response in both regions during the 600- to 720-min interval. All t values > 2.54; *, $P < .05$, **, $P < .01$.

significant decline in nucleus accumbens DA may reflect this difference in sensitivity to depletion. However, it is important to note that DA depletion probably does not play a critical role in the behavioral profile associated with multiple runs because no significant depletion was observed after multiple runs with the lowest doses of AMPH and METH tested. Furthermore, the degree of depletion that we did observe in the caudate-putamen is considerably less than has been previously reported during runs with similar doses of METH (O'Dell *et al.*, 1991; Weihmuller *et al.*, 1992) indicating, as has previously been suggested (Schmidt *et al.*, 1985a, 1985b; Segal and Kuczenski, 1997; Stephans and Yamamoto, 1996), that relatively low-dose METH pretreatment affords significant protection against the depleting effects of subsequent high-dose METH administration. In addition to protection against DA depletion, as well as against the toxic sympathomimetic effects (Angrist, 1994b; Fischman *et al.*, 1985; Fischman and Schuster, 1974; Schmidt *et al.*, 1985b) of high-dose stimulant administration, it is likely that lower-dose pretreatment regimens alter the response of other neurochemical systems to subsequent high-dose stimulant runs. For example, lower-dose METH pretreatment substantially attenuated the rise in caudate-putamen extracellular glutamate associated with a binge pattern of high-dose METH administration, without altering the caudate-putamen DA response (Stephans and Yamamoto, 1996). These differential changes in responsivity resulting from pretreatment exposure may have important implications for the behavioral responses associated with binge stimulant administration and their potential relevance to stimulant abuse.

In contrast to the DA responses, the extracellular 5-HT response was differentially affected by run exposures to the two drugs (*i.e.,* both brain regions exhibited a decrease in 5-HT response relative to the first injection with successive injections of METH, but neither region significantly changed with AMPH). Because our previous results (Segal and Kuczenski, 1997) revealed a pronounced tolerance/tachyphylaxis of the 5-HT response in both brain regions with 8.0 mg/kg AMPH runs, the progressive decrement in the 5-HT response appears to be dose dependent. Furthermore, the decrement in response with multiple injections of METH but not the equimolar dose of AMPH may reflect a depletion of a relevant pool of this transmitter because METH appears to be more potent than AMPH in releasing 5-HT (fig. 8; see also Kuczenski *et al.*, 1995).

The relative AMPH- and METH-induced changes in extracellular 5-HT are not consistent with our previous suggestions that stimulant-induced alterations in 5-HT play an important role, especially in the qualitative features of the stereotypy response (Kuczenski and Segal, 1989; Segal, 1976, 1977). That is, the drug-related differences in 5-HT are most pronounced after the initial injection of the run and diminish

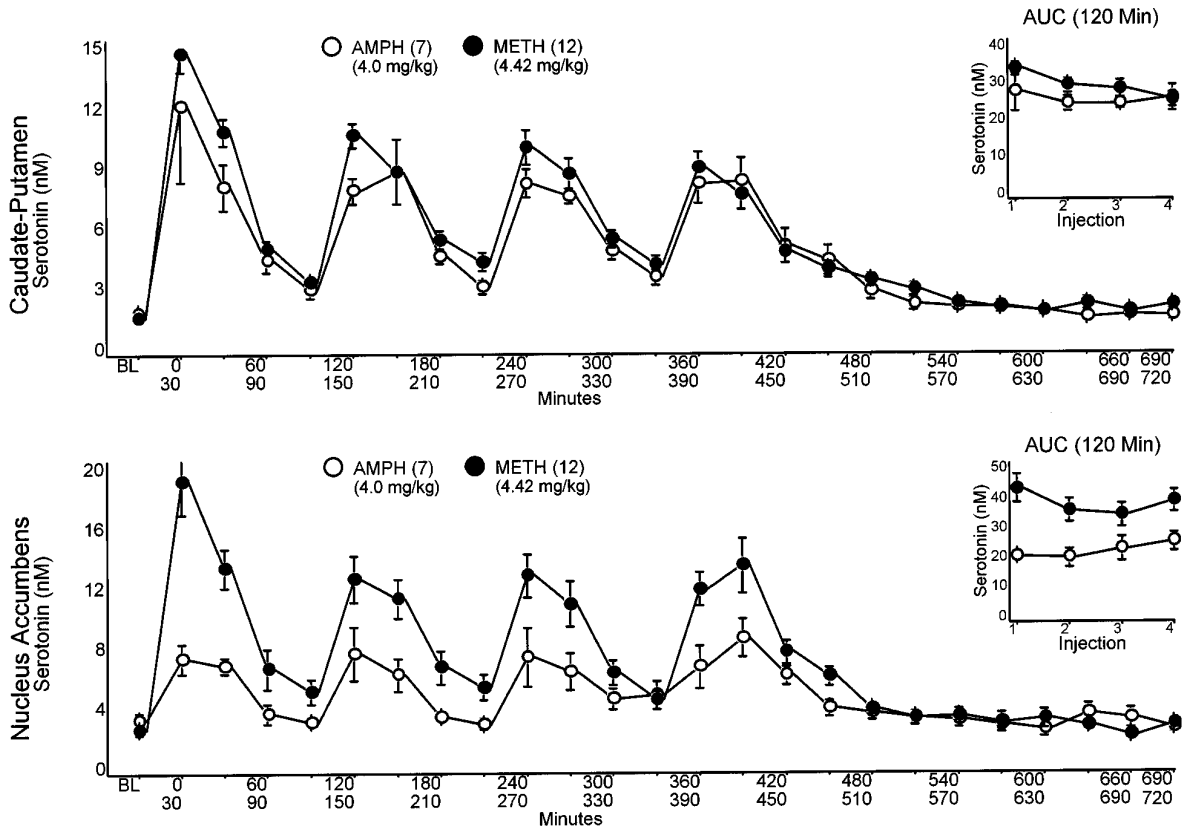

Fig. 8. Extracellular 5-HT in caudate-putamen (top) and nucleus accumbens (bottom) during the first run of four successive injections at 2-hr intervals of AMPH (4.0 mg/kg) or METH (4.42 mg/kg). Insets, AUC of the 5-HT response during the 120-min interval after each injection. ANOVA revealed a significant effect of injection for METH in both caudate-putamen ($F = 14.8$, $P < .001$) and nucleus accumbens ($F = 3.92$, $P < .05$) but not for AMPH in either brain region.

TABLE 1

Tissue levels of METH and AMPH after METH administration

Groups of animals were pretreated with SDI METH or SDI METH plus runs or an equivalent number of saline injections (as described in Methods) and then challenged with METH. Animals were killed 3.5 hr after the last METH injection.

Pretreatment	METH treatment	Caudate-putamen		Frontal cortex	
		AMPH	METH	AMPH	METH
	mg/kg	*nmol/g*			
Saline	4.42	1.18 ± 0.23	2.06 ± 0.08	1.41 ± 0.17	1.72 ± 0.12
SDI × 6	4.42	1.44 ± 0.64	1.99 ± 0.07	1.48 ± 0.10	1.76 ± 0.12
SDI × 6	4 × 4.42	2.18 ± 0.36[a]	3.54 ± 0.35[a]	3.10 ± 0.10[ab]	3.57 ± 0.13[a]
SDI × 6 + Run × 5	4 × 4.42	2.14 ± 0.22[a]	3.54 ± 0.35[a]	3.92 ± 0.52[ab]	3.91 ± 0.49[a]

[a] $P < .01$ compared with saline pretreatment (all $t > 3.88$).
[b] $P < .02$ compared with caudate-putamen (all $t > 2.83$).

with subsequent drug injections, whereas the stereotypy profiles become most distinguishable in terms of qualitative features with successive injections (*i.e.,* METH- but not AMPH-treated animals developed self-directed oral behaviors; data not shown). Therefore, it is presently unclear how the differences in the 5-HT response during AMPH and METH runs contribute to their behavioral profiles.

Examination of the behavioral responses to AMPH and METH reveals quantitative features, only some of which appear to parallel the relative effects of these drugs on extracellular DA. For example, the marked prolongation of the poststereotypy locomotor phase that occurs only after METH run exposure is paralleled by higher extracellular DA levels in both caudate-putamen and nucleus accumbens for the METH compared with the AMPH responses. It is likely that pharmacokinetic factors contribute to the prolongation of both the DA and the behavioral responses because brain levels of METH and its active metabolite, AMPH, were each ~2-fold higher than observed in response to either an acute or a seventh SDI METH treatment (table 1). In contrast, during AMPH run exposure, AMPH levels increased by only ~20% (Segal and Kuczenski, 1997). One possible explanation for this difference is that METH metabolism and elimination may change during the run because the METH levels after run exposure are substantially higher than would be predicted (Benet *et al.*, 1990) based on the half-life of METH (Melega *et al.*, 1995).

The role that differences in drug metabolism might play in the abuse of these drugs is difficult to extrapolate from the present studies. However, it is not likely that such pharma-

cokinetic changes are involved in the behavioral response that emerges with multiple daily runs because there were no further changes in METH metabolism after the first run (table 1), whereas the most profound changes in the behavioral response were not apparent until repeated run exposure.

In conclusion, the results of this study further support our hypothesis that relatively gradual exposure to stimulants (*i.e.,* escalating doses or SDI, followed by repeated runs) results in a unique behavioral and neurochemical profile that cannot be produced by any acute or SDI dose tested. One of the most obvious features of the altered behavioral profile is the profound change in the relationship between the continuous stereotypy and locomotor components of the response. Because this behavior also appears to be qualitatively different from the enhanced poststereotypy locomotor response that develops after SDI, the further increase in the magnitude of the locomotion resulting from run exposures may not simply reflect a further augmented responsiveness (*i.e.,* greater sensitization). It is also important to note that the altered behavioral profile can be elicited in response to a subsequent single injection challenge, indicating that these changes are not simply a function of cumulative dose.

On the basis of these results, we suggest that if stereotypy represents a coping mechanism, as previously proposed (Mason, 1991; Mittleman *et al.*, 1991), the multiple run-induced behavioral profile may reflect a state of stress or hyperarousal, relatively unattenuated by the expression of response perseveration as a "calming" mechanism (Davis and Schlemmer, 1980; Rylander, 1969, 1980; Schiorring, 1977). Therefore, rather than reflecting an enhanced responsiveness of the neurochemical systems underlying the locomotor component of the response, it is conceivable that mechanisms mediating response perseveration are impaired as a function of multiple run exposures. Although further research will be required to determine the likely complex neurochemical and behavioral processes involved, our results support and extend our previous findings (Segal and Kuczenski, 1997) and provide additional evidence for the potential significance of these profound alterations for the effects of high-dose stimulant abuse.

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