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Amphetamine-Induced Locomotor Stereotypy in Rats Is Reduced by a D_1 but not a D_2 Antagonist

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FRITTS, M. E., K. MUELLER AND L. MORRIS. *Amphetamine-induced locomotor stereotypy in rats is reduced by a D_1 not a D_2 antagonist.* PHARMACOL BIOCHEM BEHAV **58**(4) 1015–1019, 1997.—Amphetamine produces locomotor stereotypy (repetitive routes of locomotion) in an open field. In this research we tested the ability of the D_1 antagonist SKF 83566 and the D_2 antagonist sulpiride to block the locomotor stereotypy produced by 2 mg/kg amphetamine. SKF 83566 decreased amphetamine-induced locomotor stereotypy; sulpiride had no consistent effect on amphetamine-induced locomotor stereotypy. There was no evidence that either antagonist potentiated the effect of the other. © 1997 Elsevier Science Inc.

Amphetamine Stereotypy D_1 and D_2 antagonists

AMPHETAMINE, an indirect dopamine agonist, reliably produces hyperlocomotion and focused stereotypy (hypolocomotion accompanied by intense sniffing/licking/biting of a restricted area of the environment) in rats (14,16). Traditionally, amphetamine-induced hyperlocomotion and focused stereotypy have been considered separately. Recently, however, several laboratories have become interested in amphetamine-induced "locomotor-stereotypy"—amphetamine-induced repetition of particular routes of locomotion in an open field (see Fig. 1). This article addresses the role of dopamine D_1 and D_2 receptors in producing amphetamine-induced locomotor stereotypy.

Amphetamine-induced hyperlocomotion is mediated by increased release of dopamine in nucleus accumbens (3); many components of amphetamine-induced focused stereotypy are mediated by increased release of dopamine in striatum (8). Therefore, dopamine is a likely candidate for mediating amphetamine-induced locomotor stereotypy. This hypothesis is supported by the observation that haloperidol (a D_1/D_2 antagonist) reduces amphetamine-induced locomotor stereotypy in an open field (10). In addition, the direct dopamine agonist apomorphine produced repetitive locomotion in smaller activity chamber (4).

However, not all data consistent with the hypothesis that dopamine mediates amphetamine-induced locomotor stereotypy in an open field. A wide range of doses of apomorphine failed to produce locomotor stereotypy in an open field (12). Furthermore, scopolamine (a cholinergic antagonist) also produced locomotor stereotypy (12).

Therefore, the involvement of dopamine in locomotor stereotypy has yet to be firmly established. A related question is the relationship between locomotor stereotypy and amphetamine-induced behaviors. On one hand, locomotor stereotypy could simply be another manifestation of focused stereotypy (or by the same neurochemical mechanism that produces hyperlocomotion). In such a case, studying locomotor stereotypy would provide no more information than obtained by studying the more "traditional" behavioral effects of amphetamine.

On the other hand, locomotor stereotypy could be distinct (both behaviorally and neurochemically) from hyperlocomotion and focused stereotypy. This latter hypothesis is supported by the failure of apomorphine to produce locomotor stereotypy in an open field. In addition, the atypical neuroleptic clozapine reduced amphetamine-induced hyperlocomotion without affecting locomotor stereotypy (10). Clozapine either fails to affect or actually increases amphetamine-induced focused stereotypy (13). Finally, although haloperidol reduces amphetamine-induced locomotor stereotypy, the dose–response relationship is different for locomotor stereotypy than for hyperlocomotion and focused stereotypy (10).

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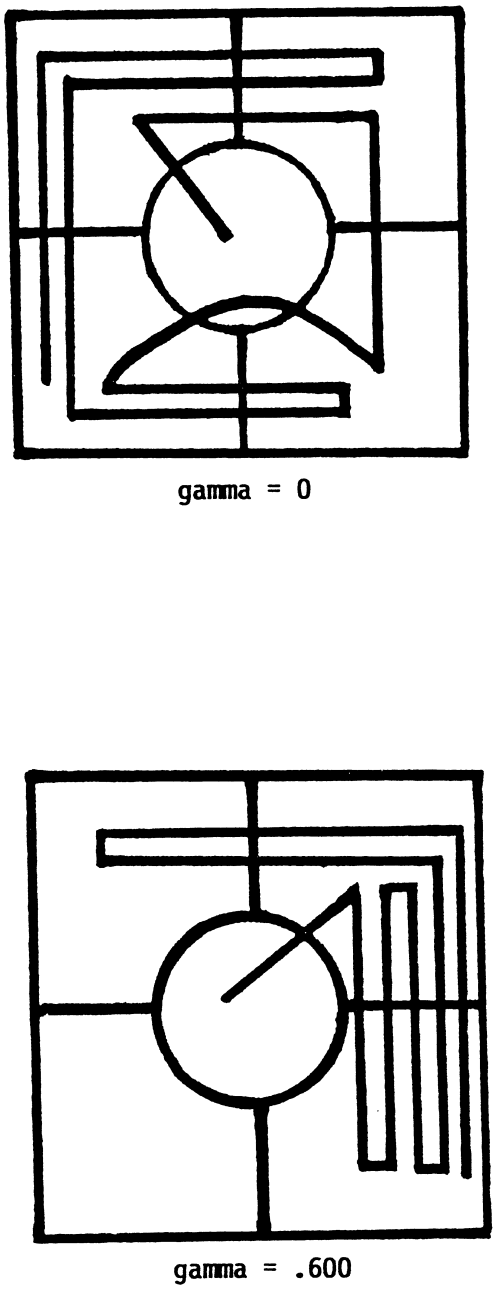

FIG. 1. Schematic of a rat's path through the open field. The path is drawn spiraling outward to simplify data reduction; most rats locomote near the walls of the open field. The lower schematic represents locomotor stereotypy.

One way to assess the role of dopamine in amphetamine-induced locomotor stereotypy and to assess the relationship between locomotor stereotypy is to use more specific dopaminergic drugs (1,2,7). The discovery of different dopamine subreceptors (D_1, D_2, D_3, D_4, D_5, and D_6) has facilitated efforts in distinguishing between amphetamine-induced behaviors such as focused stereotypy and hyperlocomotion (1,9,13). In this research, we tested the ability of SKF 83566 (D_1 antagonist) and of sulpiride (D_2 antagonist) to block amphetamine-induced locomotor stereotypy. We were particularly interested in the possibility that hyperlocomotion and locomotor stereotypy would be affected differently by either of the two antagonists.

METHOD

Animals

Male Wistar rats (350 to 450 g), bred in the departmental animal facilities, were housed individually in standard wire-mesh cages on a 12-h light/dark cycle. Food and water were available ad lib; rats were tested at 4 h prior to lights out.

Apparatus and Data Reduction

An open field (112 × 112 × 30 cm) was divided into five equal areas (a center and four surrounding areas). A video camera was mounted approximately 170 cm above the floor of the open field. Each animal was videotaped for 1 h; the animal's route through the open field was recorded by a videotracking system (Videomex-V, Columbus Instruments).

Trained assistants then determined the values of the dependent variables. To calculate the measure of locomotor stereotypy, gamma, the rat's route through the open field was divided into a series of trips. Briefly [for more information see (11)], gamma quantifies the probability that the rat will repeat the same trip that it just has exhibited. Gamma values range from 0 (no repeated trips) to 1.0 (the same trip is repeated throughout the time interval). Rats that fail to locomote during a time interval are assigned "missing data" for gamma during that interval.

Statistically significant differences between groups were assessed with BMDP 5V (15). This program is specifically designed for repeated measures ANOVA with missing data. It reports a Wald test of significance, which is in turn based on the chi-square distribution.

The number of lines crossed was determined in the usual way. Statistically significant differences between groups were assessed with ANOVA, BMDP 2V (5).

Testing

Each animal was briefly handled once a day for 5 days prior to testing. All animals were then habituated to the open field for 40 min on 2 consecutive days prior to testing. On the day of testing, each rat was first injected with the antagonist of interest and was returned to its home cage for 30 min. Amphetamine-sulfate (Sigma, 2 mg/kg—dose calculated as the salt) was then injected and the rat was immediately placed in the center of the open field. Each rat was tested only once. All injections were subcutaneous.

Experiments

The first pair of experiments assessed the effects of the D_1 dopamine antagonist SKF 83566 (Research Biomedicals, Inc.; saline, 0.005 mg/kg, 0.01 mg/kg, 0.03 mg/kg; $n = 8$ per group) or the D_2 dopamine antagonist sulpiride (Research Biomedicals, Inc.; saline, $n = 11$; 15 mg/kg, $n = 9$; 25mg/kg, $n = 9$) on amphetamine-induced changes in gamma and lined crossed. The design for each experiment was a two-factor (dose × time) ANOVA. Each dependent variable was analyzed separately.

The third experiment combined the effects of SKF 83566 plus sulpiride on amphetamine-induced changes in gamma and lines crossed. Four doses of SKF 83566 (0.005 mg/kg, 0.075 mg/kg, 0.01 mg/kg, 0.03 mg/kg) and two doses of sulpir-

ide (15 mg/kg and 25 mg/kg) were tested in a 4 (dose of SKF) × 2 (dose of sulpiride) × 6 (10-min intervals after injection) factorial design (n = 8 per group). Again, each dependent variable was analyzed separately.

RESULTS AND DISCUSSION

Sulpiride

Sulpiride alone failed to block amphetamine-induced locomotor stereotypy (see Fig. 2). In fact, on the surface sulpiride seemed to enhance gamma scores. However, this effect was not statistically significant, $\chi^2(2) = 1.65$, *NS*. Furthermore, the gamma scores of the saline-amphetamine controls were somewhat lower than usual in this experiment. Testing higher doses of sulpiride was fruitless—higher doses (45 mg/kg, 65 mg/kg) were so effective at reducing amphetamine-induced locomotion that insufficient locomotion remained for calculation of gamma scores (data not shown).

The failure of sulpiride to block amphetamine-induced locomotor stereotypy was surprising (6). Haloperidol is far more effective at blocking D_2 receptors than D_1 receptors, and haloperidol is very effective at blocking amphetamine-induced locomotor stereotypy. Apparently, the D_1-blocking properties of haloperidol were responsible for blocking locomotor stereotypy.

Unlike locomotor stereotypy, both doses of sulpiride reduced amphetamine-induced hyperlocomotion, $F(15,120) = 2.375$, $p < 0.05$, for the main effect of time. The differences appear to be minor (see Fig. 2).

SKF 83566

SKF 83566 significantly reduced amphetamine-induced locomotor stereotypy (see Fig. 3). In comparison to saline + amphetamine controls, locomotor stereotypy was reduced by the highest dose of SKF 83566; however, lower doses were ineffective. The main effect of dose after injection was significant, $\chi^2(3) = 24.784$, $p < 0.05$, as was the main effect of time of SKF 83566, $\chi^2(5) = 29.834$, $p < 0.05$. The interaction was not significant.

As shown in Fig. 3, SKF 83566 produced a dose-dependent suppression of amphetamine-induced hyperlocomotion, $F(15, 40) = 1.91$, $p < 0.05$ for the dose × time interaction). SKF 83566 was more effective at reducing the number of lines crossed over time, but only in the early time periods and only in the higher doses.

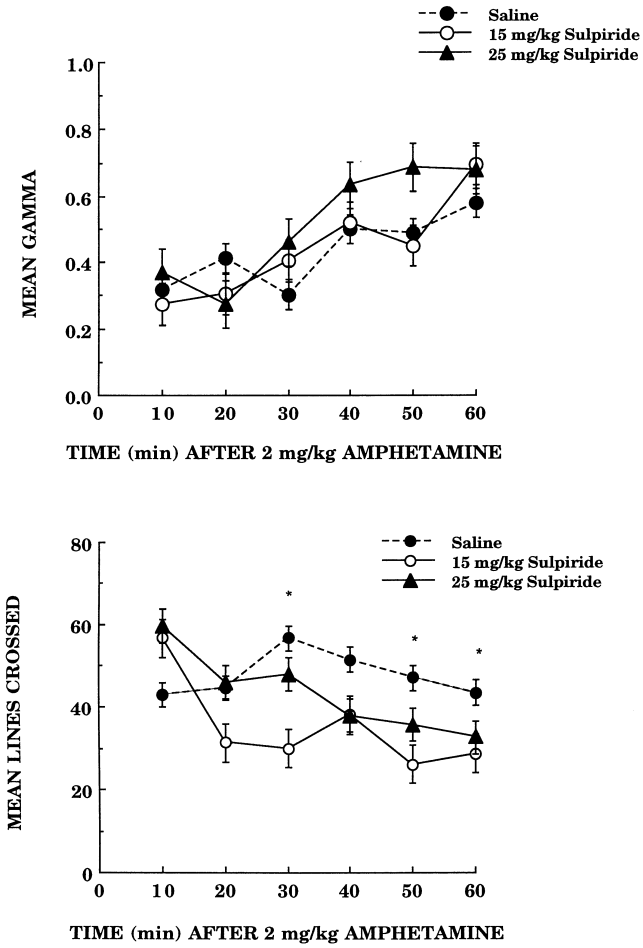

FIG. 2. The effects of the D_2 antagonist sulpiride on locomotor stereotypy (top) and hyperlocomotion (bottom) produced by 2 mg/kg amphetamine.

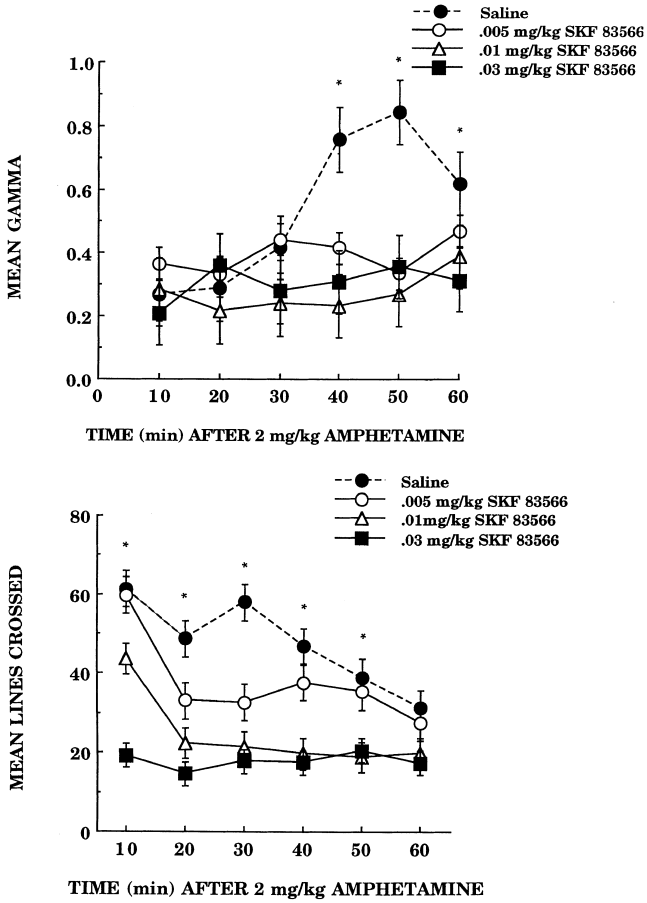

FIG. 3. The effects of the D_1 antagonist SKF 83566 on locomotor stereotypy (top) and hyperlocomotion (bottom) produced by 2 mg/kg amphetamine.

SKF 83566 + Sulpiride

Although some combinations of doses of SKF 83566 + sulpiride reduced amphetamine-induced locomotor stereotypy, $F(30,360) = 3.46$, $p < 0.05$ for the dose × time interaction, there was no indication that either drug potentiated the effect of the other (see Fig. 4). Except for 0.0075 mg/kg SKF 83566 + 25 mg/kg sulpiride, the effect of SKF 83566 + sulpiride was generally similar to that of the appropriate dose of SKF 83566 alone.

The data on lines crossed were similar to the gamma data. That is, although several combinations of doses of SKF 83566 and sulpiride reduced amphetamine-induced hyperlocomotion, there was no indication that either drug potentiated the effect of the other (see Fig. 5). For example, note that the data from 0.01 mg/kg SKF 83566 + 15 mg/kg sulpiride are very similar to the data from 0.01 mg/kg SKF 83566 + 25 mg/kg sulpiride. Likewise, 0.03 mg/kg SKF 83566 + 25 mg/kg sulpiride was no more effective than 0.03 mg/kg SKF 83566 + 15 mg/kg sulpiride.

In addition, comparing Figs. 3 and 4 indicate that 30 min after administration of amphetamine, 0.005 mg/kg SKF 83566 reduced hyperlocomotion by about 50% (Fig. 3); 0.005 mg/kg SKF 83566 + 25 mg/kg sulpiride also reduced hyperlocomotion by about 50% (Fig. 4). Again, there is no evidence of a synergistic effect of these two drugs.

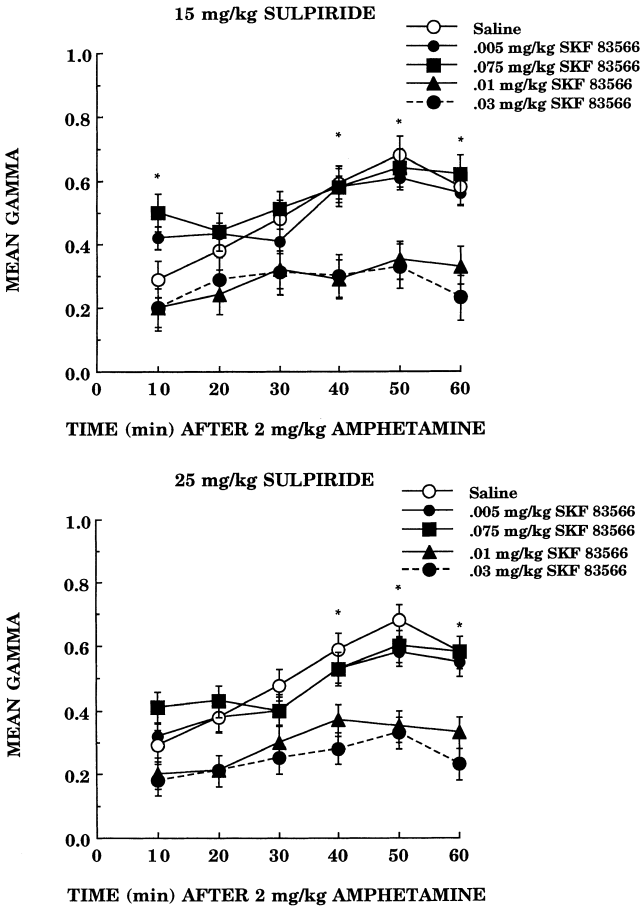

FIG. 4. The effects of combined pretreatment with sulpiride and SKF 83566 on locomotor stereotypy produced by 2 mg/kg amphetamine. For ease of comparison, saline + amphetamine data are included in both the top and bottom graphs.

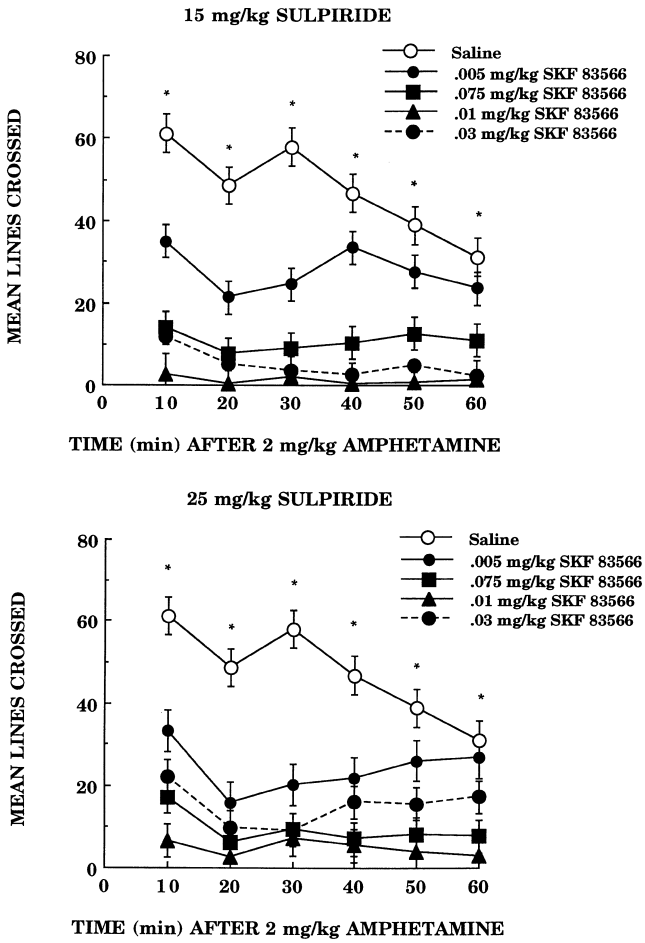

FIG. 5. The effects of combined pretreatment with sulpiride and SKF 83566 on hyperlocomotion produced by 2 mg/kg amphetamine. For ease of comparison, saline + amphetamine data are included in both the top and bottom graphs.

CONCLUSIONS

The main findings of these experiments were 1) the D_1 dopamine antagonist SKF 83566 was far more effective than the D_2 dopamine antagonist sulpiride at blocking amphetamine-induced locomotor stereotypy and hyperlocomotion; 2) there was no evidence that either antagonist potentiated the effect of the other. The implication of these results is that locomotor stereotypy and hyperlocomotion are at least partly independent behavioral phenomena. Locomotor stereotypy may thus be more closely related to focused stereotypy than to hyperlocomotion. The possibility that D_3, D_4, D_5, or D_6 dopamine subreceptors are also involved in the production of amphetamine-induced locomotor stereotypy needs further investigation as well as dopamine's known interaction with cholinergic mechanisms.

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REFERENCES

1. Braun, A. R.; Chase, T. N.: Obligatory D1/D2 receptor interaction in the generation of dopamine agonist-related behaviors. Eur. J. Pharmacol. 131:301–306; 1988.
2. Clark, D; White, F. J.: Review: D1 dopamine receptor—The search for a function: A critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse 1:347–388; 1987.
3. Creese, I.; Iverson, S.: The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. Psychopharmacology (Berlin) 39:345–357; 1974.
4. Geyer, M. A.; Russo, P. V.; Segal, D. S.; Kuczenski, R.: Effects of apomorphine and amphetamine on patterns of locomotor and investigatory behavior in rats. Pharmacol. Biochem. Behav. 28: 393–399; 1987.
5. Jennrich, R.; Sampson, P.; Frane, J.: Analysis of variance and covariance including repeated measures. BMDP statistical manual. Berkeley, CA: University of California Press; 1985.
6. Kenyon, P.; Moore, S.; Hampson, J.: Effect of sulpiride on amphetamine-induced activity and stereotypyed locomotion. Curr. Psychol. Res. Rev. 11:241–253; 1992.
7. Kebabian, J. W.; Calne, D. B.: Multiple receptors for dopamine. Nature 277:93–96; 1979.
8. Kelly, P. H.; Seviour, P. W.; Iversen, S. D.: Amphetamine and apormorphine responses in following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Brain Res. 94:507–522; 1975.
9. Moore, N. A.; Axton, M. S.: The role of multiple dopamine receptors in apomorphine and N-n-propylnorapomorphine-induced climbing and hypothermia. Eur. J. Pharmacol. 178:195–201; 1990.
10. Mueller, K.: Locomotor stereotypy is produced by methylphenidate and amfonelic acid and reduced by haloperidol but not clozapine or thioridazine. Pharmacol. Biochem. Behav. 45:71–76; 1993.
11. Mueller, K.; Hollingsworth, E. M.; Cross, D. R.: Another look at amphetamine-induced stereotyped locomotor in rats using a new statistic to measure locomotor stereotypy. Psychopharmology (Berlin) 97:74–79; 1989.
12. Mueller, K.; Peel, J.: Scopolamine produces locomotor stereotypy in an open field but apomorphine does not. Pharmacol. Biochem. Behav. 36:613–617; 1990.
13. Robertson, A.; MacDonald, C.: Atypical neuroleptics clozapine and thioridazine enhance amphetamine-induced stereotypy. Pharmacol. Biochem. Behav. 21:97–101; 1984.
14. Schiorring E.: An open field study of stereotyped locomotor activity in amphetamine-treated rats. Psychopharmacology (Berlin) 66:281–287; 1979.
15. Schlucter, M. D.: Unbalanced repeated measures models with structured covariance matrices. In: Dixon, W. J.; Brown, M. B.; Engelman, L.; Jennrich, R. I., eds. BMDP statistical software manual, vol. 2. Berkeley, CA: University of California Press; 1990: 1207–1244.
16. Segal, D.: Behavioral characterization of *d*- and *l*-amphetamine: Neurochemical implications. Science 190:475–477; 1975.