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Amphetamine-Induced Stereotypic Responses in Roman High- and Roman Low-Avoidance Rats[1]

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DRISCOLL, P., I. LIEBLICH AND E. COHEN. *Amphetamine-induced stereotypic responses in Roman high- and Roman low-avoidance rats.* PHARMACOL BIOCHEM BEHAV **24**(5) 1329–1332, 1986.—The effects of 0, 1, 3 and 5 mg/kg d, l-amphetamine (AMPH) on male RHA/Verh and RLA/Verh rats were measured, at 25 and 55 min post-injection, using an observational method in which six categories of behavior were scored. RHA/Verh rats displayed a more rapid increase of AMPH-induced stereotyped behavior, mostly due to drug-related differences in scanning (head-bobbing), whereas the differences in rearing seen (RHA/Verh>RLA/Verh) were attributable to purely genetic effects. These results were compared with those of previous experiments which measured apomorphine-induced stereotyped behavior in these, and other, Roman lines. It was concluded that the RHA/Verh rats probably showed a stronger response to AMPH due to their higher, and more drug-responsive, striatal dopamine turnover rate.

Amphetamine Genetic selection Pharmacogenetics Roman high- and low-avoidance rats
Psychogenetics Stereotyped behavior Striatal dopamine Apomorphine

DERIVED from the original Roman high- and low-avoidance rat lines [5], the RHA/Verh and RLA/Verh rat lines, bred at the colony located in Lausanne, Switzerland, are selected on the basis of rapid acquisition of the two-way conditioned avoidance response versus, respectively, the failure to acquire that response. This selection process has led to further differences in other behavioral measures, especially several which have indicated that the RLA/Verh rats are more "emotional," as well as in neurochemical parameters [9]. It has been of particular interest to note that the RHA/Verh rats have a higher striatal turnover rate of dopamine (DA) (e.g., [10,11]) and that they are also more affected by the same doses of amphetamine (AMPH) in certain behavioral tests, than are RLA/Verh rats [2,8].

In the light of those findings, and given that RHA/Verh rats, as well as other RHA rats, have exhibited an accelerated onset of apomorphine-induced stereotyped behavior in comparison to their respective counterparts [12,13], the present study has investigated the development of AMPH-induced stereotyped behavior in RHA/Verh and RLA/Verh rats. Not only was it predicted that the RHA/Verh rats would show a stronger drug-related response than would the RLA/Verh rats, but the method of measurement used allowed for the fragmentation of that response into several, separately observed categories of behavior, making it possible to pinpoint the differential effects of both AMPH, and genetic selection, on each of those separate categories.

METHOD

Eighteen naive, male RHA/Verh and 18 naive, male RLA/Verh rats, all five months old at the time of the experiment, were used. The rats had been housed three to a cage in an air-conditioned room maintained at 23°C, on a 12:12 hr light-dark cycle (lights on at 0730), with food and water ad lib.

Each rat was observed and rated on four different days for stereotyped behavior, after an IP injection of each of the four doses of AMPH (0, 1, 3 and 5 mg/kg d,l-amphetamine sulphate, dissolved in physiological saline). All animals were tested every second day, with the order of the (coded) doses scrambled differently for each rat. The rats were observed by two observers, four at a time, in four separate semicircular (60 cm dia.) Plexiglas cages with shavings covering each floor. All observations were conducted between 0900 and 1300 hr.

Each batch of four rats was placed into the observation cages twice during a session, at 20 and 50 min post-injection,

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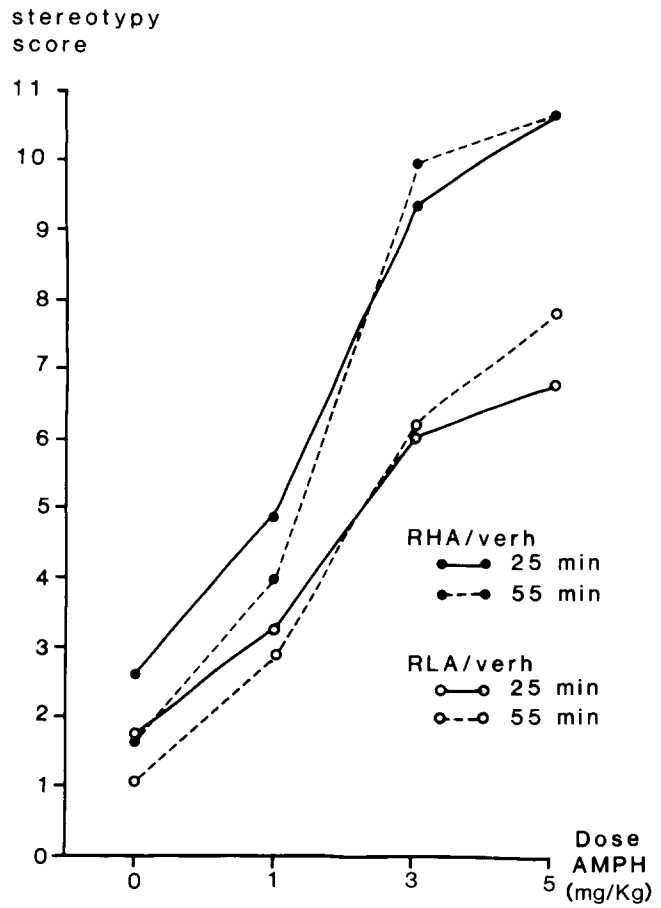

FIG. 1. The total stereotypy scores for both rat lines, compared in relation to amphetamine dosage and post-injection observation time.

for 10 min. They were given a five min adaptation period, after which they were rated during a subsequent five min period, first for rearing behavior (one to two min), and then for the other types of behavior. The person preparing and injecting the animals did not participate in the observation procedure, and the raw data, arrived at by consensus, were placed onto pre-prepared rating sheets.

The method of rating was adapted from the one developed by Goldman [17]. The scale was designed to rate specific behavioral elements amplified by AMPH, as well as the appearance of patterns of these elements. Stereotypy was rated by the degree to which the rat displayed repetitive (a) rearing, (b) rhythmic scanning (also referred to in the literature as "head-bobbing, -swaying or -waving"), (c) "nail-biting" (with or without face-washing, but certainly not "grooming" as the term is commonly understood), (d) forward, backward, or rotational locomotion, and (e) licking of the walls of the cage. An additional category, (f) pattern of stereotyped behavior, was also included, in order to realistically evaluate those animals whose stereotypy was so extremely pronounced as to virtually narrow their behavioral repertoire to a regular and predictable combination of two of the above behavioral elements (one of which was, incidentally, always scanning). All six items were graded from one to four, in terms of their frequency and duration of appearance.

TABLE 1

SEPARATE SCORES FOR EACH OF THE BEHAVIORAL ITEMS MEASURED, WITH THE TWO OBSERVATION PERIODS COMBINED

Behavior	Rat Line	Dose Amphetamine (mg/kg) 0	1	3	5
Scanning	RHA/Verh	0.79	1.80	3.36	3.39
	RLA/Verh	0.66	1.14	2.17	3.08
Rearing	RHA/Verh	0.94	1.42	2.42	2.50
	RLA/Verh	0.55	0.92	1.53	1.50
Locomotion	RHA/Verh	0.28	1.11	1.94	1.33
	RLA/Verh	0.14	0.83	1.72	1.47
Nail-biting	RHA/Verh	0.00	0.03	0.06	0.14
	RLA/Verh	0.00	0.08	0.06	0.33
Wall-licking	RHA/Verh	0.00	0.06	0.33	0.36
	RLA/Verh	0.03	0.03	0.00	0.03
Pattern	RHA/Verh	0.00	0.03	1.58	2.80
	RLA/Verh	0.00	0.06	0.67	1.05

RESULTS

ANOVAs for repeated measures were employed to assess the effects of genetic background (line), AMPH doses (dose) and the two observation periods in each session (time) on stereotyped behavior. A significance criterion of $p<0.05$ was pre-chosen. For each rat, the scores across the various behavioral categores observed were summed up for each period within each session, yielding the stereotypy scores, the means of which are shown in Fig. 1 for the two genetic lines, four AMPH doses and the two time intervals.

A significant line effect was seen, $F(1,34)=42.50$, as well as a significant dose effect, $F(3,102)=116.32$, but no significant time effect. A significant line × dose interaction, $F(3,102)=4.92$, as well as a significant dose × time interaction, $F(3,102)=4.34$, were also found. It was thereby established that the RHA/Verh rats showed a more rapid increase in stereotyped behavior, in response to AMPH, than did the RLA/Verh rats, and that this difference became more accentuated at higher dose levels, irrespective of the observation interval used.

In addition to the total score analysis, possible line differences were analyzed in regard to the most common behavioral categories seen, i.e., ANOVAs for repeated measures were conducted on rearing, scanning and locomotion. The values for these items, as well as for the others, with the two observation periods combined, are shown in Table 1. Significant line, $F(1,34)=18.15$, dose, $F(3,102)=25.93$, and time, $F(1,34)=6.51$, effects were obtained in respect to rearing, but no significant interactions were found, indicating that even RHA/Verh control rats (0 mg/kg AMPH) reared more than RLA/Verh control rats did, and that this activity increased about equally in both lines with increasing dosage.

Significant line, $F(1,34)=24.29$, and dose, $F(3,102)=85.18$, effects, as well as significant line by dose, $F(3,102)=3.23$, and dose by time, $F(3,102)=7.09$, interactions were obtained with respect to scanning, indicating that AMPH had a more pronounced effect in the RHA/Verh rats than in the RLA/Verh rats in this category. The analysis of locomotion revealed only dose, $F(3,102)=50.02$, and time, $F(1,34)=12.19$, effects, and a dose by time, $F(3,102)=4.41$, interaction.

DISCUSSION

This experiment has demonstrated a more rapid increase of AMPH-induced stereotyped behavior in RHA/Verh rats, as compared to RLA/Verh rats. In addition, most of this effect has been determined to be due to differences seen in AMPH-induced rhythmic scanning (especially when the dominant role of that behavioral item in the "pattern" category is also taken into account). In comparison, the large differences in rearing behavior which were seen throughout the study appeared to be due to initial genetic differences which were merely amplified by the drug. In that regard, it should be noted that the more frequent rearing activity observed with the control RHA/Verh rats in the present study is consistent with observations made several years ago on the original RHA and RLA lines of rats [16,18].

As previously mentioned, RHA/Verh rats have also been seen to be more affected than RLA/Verh rats by small doses of AMPH in other behavioral tests [2,8], and similar results have been consistently found following injections of small doses of nicotine [2,3]. In addition, both male RHA/Verh and RHA/iop rats have shown a more rapid onset of apomorphine (APO)-induced stereotypy than have their RLA/Verh and RLA/iop counterparts [12,13]. The results of these latter experiments suggest some additional interesting comparisons between AMPH-induced, and APO-induced, stereotypy.

First of all, the APO study with the London-based Roman strains [13] was conducted over a 50 min post-injection period, as was the present AMPH experiment, and was also concerned, essentially, with the development of stereotypy. Those authors recognized that fact and actually stated that whereas the stereotyped behavior of RLA/iop rats appeared to be still increasing at the end of that period, that of the RHA/iop rats seemed to have already reached its peak and was starting to diminish. This phenomenon, i.e., whereas RHA rats show a more pronounced APO-induced stereotypy during the early stages but RLA rats show a longer lasting drug effect, was confirmed in experiments with RHA/Verh and RLA/Verh rats, which were continued for 90 min post-injection. Therein the hypothermic effects of APO were also found to be longer-lasting in RLA/Verh rats [12]. However, whereas the stereotypic effects of APO generally disappear within 90 min, the sterotypic effects of AMPH may last as long as 3–6 hr [6]. Although it is possible that RLA/Verh rats might have also shown longer-lasting AMPH effects, due to a lower drug-metabolizing capability, it was technically impossible in the present study to extend the sessions for that length of time, due to limited access to the observation cages.

Other interesting variables which may be relevant to the understanding of the studies under discussion here include possible sex and species differences, as well as functional differences in various brain regions. It has been found, for example, that female rats may show a different stereotypic response than that of males [4], which was also the case in our studies concerned with APO-induced stereotypy [12]. In addition, BALB/CJ mice, which have an increased striatal DA metabolism in comparison to CBA/J mice, show more AMPH-induced stereotypy but, actually, less APO-induced stereotypy than do the latter [14]. Thus, the possibility of species differences exists. It should be noted, however, that the sites in the CNS important for stereotyped scanning, licking, biting and hyperactivity are not the same for APO and AMPH [6], and that the above-mentioned mouse strains differ from each other also in dopaminergic function in several other brain regions [14], which has not yet been determined to be the case with RHA/Verh and RLA/Verh rats.

What is known about RHA/Verh and RLA/Verh rats, however, is that the former have a higher, more drug-sensitive turnover rate of striatal DA (e.g., [10,11] and manuscript in preparation). This conforms very well to the results of the present study, as many authors, working with various strains of rats from different sources, have come to the conclusion that the striatal dopaminergic system is vital to the behavioral symptoms of AMPH-induced stereotypy in the rat [1, 4, 7, 15, 19, 22], and that AMPH produces this syndrome by increasing the amount of DA released in the striatum [4, 20, 21]. This is in contrast to the data regarding the purely locomotor effects of AMPH, the neuroanatomical origins of which are still controversial [6, 23, 25]. These locomotor effects are, incidentally, more sensitive to the lower dose ranges of AMPH than are the other effects (see Table 1, [22,25]).

Finally, the advantages of a method for the measurement of AMPH-induced stereotyped behavior, which measures several facets of that behavior separately [17], are apparent from the present study. Not only are the symptoms of AMPH toxicity in man also known to include stereotyped, repititious behaviors in regard to locomotion, the gnashing of teeth, touching and picking of the face and extremities, etc., but a recent study has shown that much more may even be learned about APO-induced stereotypy when its various components are also examined more closely, and not merely classified as "stereotypy" vs. "locomotion" [26]. Also advantageous to the present study, in the opinion of the authors, was the placing of the individual rats into the observation cages only five minutes before each rating session, which provided a realistic behavioral "background," at dose 0, against which the excitatory effects of AMPH could be measured in animals which were all equally awake.

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