Pharmacology Biochemistry & Behavior, Vol. 39, pp. 61–70. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

A Comparative Study of the Behavioral Effects of d-Amphetamine and Apomorphine in the Rat

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Received 26 July 1990

ANTONIOU, K. AND E. KAFETZOPOULOS. *A comparative study of the behavioral effects of d-amphetamine and apomorphine in the rat.* PHARMACOL BIOCHEM BEHAV **39**(1) 61–70, 1991.—A wide range of doses of d-amphetamine and apomorphine were injected into rats, in order to better characterize and compare dopaminergic agonist-induced behavioral effects. The study was carried out using a computerized technique for the quantification and analysis of various behavioral elements. Although both drugs increased motor activity and provided dose-dependent stereotyped responses, the whole pattern of behavior in the open field showed a different structure. d-Amphetamine in doses that did not produce stereotyped responses induced a wide range of varied behavioral elements with increased frequency but decreased mean duration, while apomorphine induced a more restricted behavioral profile. Furthermore, a higher frequency of freezing reaction was observed after d-amphetamine treatment in low doses but not after apomorphine treatment. Both drugs in high doses elicited a similar stereotyped syndrome characterized by repetitive movements of great duration, but at low doses the behavioral pattern was completely different. The apomorphine-induced syndrome was characterized mainly by moving and sniffing, interrupted by rearing, while the amphetamine-induced syndrome by sniffing and moving, interrupted by standing and freezing.

Amphetamine Apomorphine Open field Motor activity Behavioral pattern Rats

THE behavioral properties of psychostimulant drugs have long been the focus of intense investigation (35,39). Since the primary action of these drugs is stimulation of the central catecholamine systems, the study of them may help to elucidate these systems (45). The effects of several agonists and antagonists on the catecholaminergic systems have also been extensively analysed in experimental animals. These studies have been carried out mainly in order to investigate the dopaminergic system which is implicated in Parkinson's disease (31), schizophrenia (16,21), and in the mechanisms of action of neuroleptic drugs (16,18).

Two dopaminergic agonists, d-amphetamine (d-amp) and apomorphine (apo), are commonly used in laboratory studies. d-Amp acts as an indirect agonist enhancing the amount of neurotransmitter release (3, 8, 24) and blocking its reuptake (10,14) while apo, as a direct agonist, acts via pre- and postsynaptic dopaminergic receptors (1,12).

d-Amp exerts a well known excitatory effect on spontaneous motor behavioral elements (34,42) that is usually referred to as hyperactivity. Hyperactivity is used as a nonspecific term for increased behavioral elements (moving, sniffing, rearing, etc.) that are joined together in time. By increasing the dose the hyperactivity is replaced by stereotyped behavior (7,26). Stereotypy can be defined as the performance of an invariant sequence of species specific movements in a repetitive manner (17). On the other hand, apo has a biphasic effect. Low doses produce a depression of motor activity (44) and yawning (32). Increasing the dose of apo, hyperactivity is produced and stereotyped responses are induced with a shift from one type of stereotyped element to another, as has been suggested by Havemann et al. (23). These contrasting effects have been explained by the postulation that apo acts on different dopaminergic receptors, i.e., on presynaptically autoreceptors and postsynaptic receptors respectively (5,9).

Although it seems well documented that d-amp and apo act by increasing the dopaminergic function that mainly results in hyperactivity and stereotyped behavior, their behavioral repertoires and profiles are different, as has been supported by some authors (17, 20, 25). In order to understand these differences and to use them as a tool in the investigation of drug-induced changes, it would be necessary to estimate behavioral responses in a more accurate way, for example, by recording their frequency, as well as their duration and mean-duration, as has been suggested by a number of authors (15, 25, 36).

In the present study, therefore, a computerized technique for analyzing animal behavior has been developed which provides much more information about the behavioral profile than a mere measure of the general activity based on line crosses or photobeam interruptions, and an accuracy in the determination of the behavioral responses by recording all the components of a behavioral act. For purposes of comparison, a wide range of doses of d-amp and apo were used in order to investigate their effects

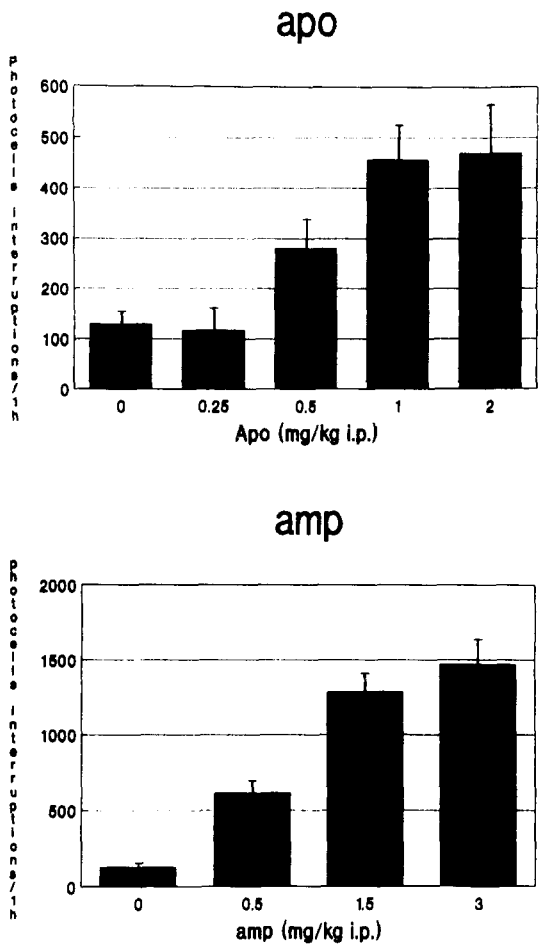


FIG. 1. Effects of saline (dose 0), d-amphetamine (amp) and apomorphine (apo) on the photobeam interruptions in a continuous 1-hour recording session in a 40 × 40 × 40 cm open field (mean + SEM).

on the animal behavior as well as possible.

METHOD

Subjects

Male Wistar rats, inbred in the Animal center of the University of Ioannina and originating from the Institute of Experimental Biology and Medicine (Borstel 2061 bei Hamburg), weighing 250–300 g, were used throughout the experiment. The rats were housed in groups of four in plastic cages with food and water freely available, under controlled illumination and temperature.

Drugs

Drugs used in the experiment were d-amphetamine sulfate and apomorphine hydrochloride (Sigma). All rats received only one drug treatment. Rats were injected IP with 0 (vehicle only), 0.5, 1.5, 3 and 6 mg/kg of d-amp and 0 (vehicle only), 0.25, 0.5, 1 and 2 mg/kg of apo. Drugs were dissolved in 0.9% saline and in the case of apomorphine, 1 mg/ml ascorbic acid was added as an antioxidant. Solutions were freshly prepared immediately prior to use. Groups of 7–10 rats were used for each drug dose.

Apparatus

The activity cage was a transparent plastic cage (40 × 40 × 40 cm) equipped with 4 photocells activated by infrared beams (2 on each side) and connected to an electronic event recorder. A white noise background was used to help screen out incidental noises during the testing time.

Behavioral Testing

The behavioral testing was performed in a sound-attenuated room with low artificial illumination between 8 a.m. and 5 p.m. Rats were habituated in the test apparatus for 30 min, then injected with d-amp, apo or saline and reintroduced immediately into the testing cage. Behavior was recorded for 1 hour, starting 15 min after the d-amp injection or immediately after the apo injection. The 1-hour session was divided into three 20-min intervals and each rat was observed for the first 10 min of every 20-min interval. During the 1-hour session cumulative counts of interruptions of the 4 photobeams were continuously recorded.

The behavior was analyzed by continuous monitoring of the animals using a technique for quantification of behavioral sequences modified after Spruijt and Gispen (43). In brief, the behavior was recorded through a video-camera by one observer using a microcomputer for data storage. A number of keys of the computer keyboard represented the behavioral elements that were tested. With the touch of the key the system clock was read and the time was stored as duration of this behavioral element. The program stored sequences of behavioral elements and their concomitantly registered time points for every 10-min observation period. The same program via a subsequent automatic analysis of the data provided the total frequency, duration and mean-duration of every element in the 10-min interval.

The behavior was noted to represent the following elements: standing, moving, sniffing, grooming, rearing, scratching, freezing, yawning, sniffing-air, head-swinging, licking. Only one of these elements (the most prominent) was scored using the keyboard. Usually only one behavioral element was exhibited, but occasionally an animal might simultaneously exhibit two. This was observed most often with sniffing, which interfered with moving, rearing or standing. In these cases, in order to facilitate the scoring, the most prominent element was scored according to the following rules:

Standing (std). The rat was on its 4 feet, essentially motionless, not moving and not actively sniffing.

Moving (mov). The rat was walking on 4 feet. Sometimes the rat was moving while sniffing the air or the apparatus and in this case sniffing was considered minimal and moving was scored.

Sniffing (sni). The rat was not moving and was smelling any part of the apparatus.

Grooming (grm). The rat was washing its face or any other part of its body, and generally its mouth was touching its body.

Rearing (rr). The rat's body was inclined vertically with its hindpaws on the floor of the activity cage and the forepaws on the wall of the cage. Sometimes the rat was rearing while sniffing, and in this case, sniffing was considered minimal and rearing was scored.

Scratching (scr). The rat was rising its hindpaw against its body.

Freezing (frz). The rat was standing on its 4 feet in a freezing position completely inactive.

Yawning (yaw). The rat was standing on its 4 feet and yawning.

Sniffing-air (sna). The rat was rearing but its forepaws were not touching any part of the activity cage.

Head-swinging (hsw). The rat was standing on 4 feet and moving its head horizontally.

Licking (lck). The rat was standing and licking any part of the apparatus.

TABLE 1

ONE-WAY ANOVA TABLE WITH DOSE AS FACTOR VERSUS FREQUENCY, DURATION AND MEAN DURATION SCORES OF 9 BEHAVIORAL ELEMENTS AFTER D-AMPHETAMINE (AMP) AND APOMORPHINE (APO) TREATMENT

	Frequency		Duration			Mean-Duration		
	F	*p*	F	*p*	*df*	F	*p*	*df*
APO								
standing	9.45	0.000	61.27	0.000	4,37	5.23	0.001	4,37
moving	11.62	0.000	16.03	0.000	4,37	4.41	0.005	4,37
sniffing	12.03	0.000	9.87	0.000	4,37	2.04	ns	4,37
grooming	5.37	0.001	3.10	0.026	4,37	1.58	ns	4,37
rearing	1.7	ns	0.95	ns	4,37	1.29	ns	4,32
scratching	4.49	0.004	3.47	0.01	4,37	1.32	ns	1,11
sniffing air	1.52	ns	2.19	ns	4,37	—	—	—
licking	4.34	0.005	5.25	0.001	4,37	—	—	—
yawning	4.58	0.004	4.85	0.003	4,37	11.82	0.001	3,16
AMP								
standing	17.59	0.001	28.18	0.001	4,58	8.35	0.001	4,58
moving	14.14	0.001	13.36	0.001	4,58	7.47	0.001	4,58
sniffing	9.78	0.001	11.70	0.001	4,58	5.83	0.001	4,58
grooming	4.50	0.003	6.79	0.001	4,58	20.01	0.001	3,49
rearing	4.94	0.002	2.02	ns	4,58	8.68	0.001	4,52
scratching	1.81	ns	1.49	ns	4,58	5.30	0.01	2,32
sniffing air	2.41	ns	1.50	ns	4,58	0.42	ns	1,20
licking	9.84	0.001	42.56	0.001	4,58	—	—	—
freezing	13.69	0.001	3.58	0.01	4,58	2.83	ns	1,36

(—): analysis not performed.

RESULTS

The data for the photocell beam interruptions and the behavioral elements tested are shown in detail in Figs. 1–5. Separate one-way analyses of variance (ANOVA) were used to test the effects of each drug (d-amp or apo) with the dose as a factor for each behavioral measure (frequency, duration and mean-duration of each behavioral element) (Table 1). In some cases where inspection of the data showed differences between the two drugs, a two-way analysis of variance was used with drug and dose as factors. Scheffé multiple range tests on group averages followed each ANOVA in order to estimate the statistical differences within each group. In addition, a factor analysis was performed with a varimax rotation of the component solution in order to analyze the behavioral effects and characterize the behavioral profiles of both drugs in more detail. Independent Student's *t*-tests were performed on every drug dose versus saline of every behavioral measure (frequency, duration, mean duration) for every behavioral element respectively, in order to provide a summary table (Table 2) of the statistically significant differences.

Photocell Interruptions

As shown in Fig. 1, d-amp induced more photocell interruptions than apo, and this was supported by the two-way ANOVA with drug and dose as factors, which revealed a significant drug effect, $F(1,104)=68.01$, $p<0.001$, as well as a significant dose effect, $F(4,104)=13.77$, $p<0.001$. Student's *t*-tests revealed significant differences for every dose versus saline ($p<0.005$) after d-amp while after apo significant differences versus saline were found after 1 and 2 mg/kg.

Standing

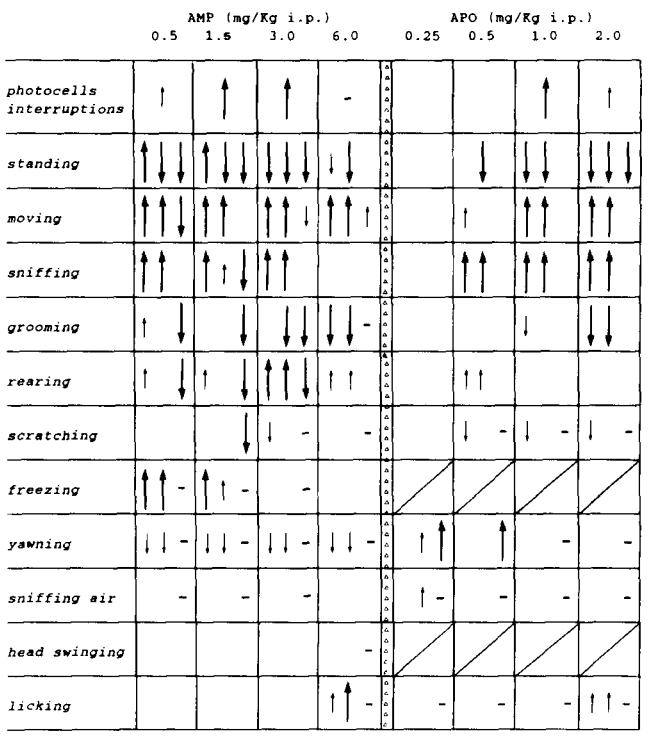
There was a significant dose effect for both drugs in frequency, duration and mean-duration of this element (Table 1: standing). Student's *t*-tests revealed that by increasing the dose of d-amp a decrease in the duration of standing was induced. The same was true after apo, except for the duration of standing after the first low dose where the effect did not reach statistical significance. The two-way ANOVA of frequency scores with dose and drug as factors revealed a significant drug effect, $F(1,104)=27.35$, $p<0.001$. Scheffé multiple range tests showed that apo induced more standing episodes than d-amp (amp-average = 182.61, apo-average = 48.85). The same analysis for duration showed a significant drug effect, $F(1,104)=15.54$, $p<0.001$. The respective Scheffé tests revealed that apo induced more standing than d-amp (amp-average = 727.31, apo-average = 906.92). The two-way ANOVA for mean-duration showed a significant drug effect, $F(1,104)=21.27$, $p<0.001$. The multiple range tests revealed that mean-duration score after apo was higher than after amp (amp-average = 4.83, apo-average = 20.15).

Moving

There was a significant dose effect after d-amp and apo in all the behavioral measures of moving (frequency, duration, mean-duration) (Table 1: moving). Student's *t*-tests revealed that apo induced significant statistical effects except for the duration and frequency after 0.25 mg/kg and for the duration of moving after 0.5 mg/kg. The same tests after d-amp revealed that increasing the dose a parallel increase of the frequency and the duration of moving was induced. Student's *t*-tests for the mean-duration showed that apo effects versus saline did not reach the statistical significance at any dose while d-amp effects showed significant differences. The two-way ANOVA on frequency and duration scores of moving with drug and dose as factors re-

TABLE 2

SUMMARY OF THE EFFECTS OF THE IP INJECTIONS OF 4 DOSES OF D-AMPHETAMINE (AMP) AND APOMORPHINE (APO) ON PHOTOBEAM INTERRUPTIONS AND 11 BEHAVIORAL ELEMENTS



At each element × dose intersection the size and the direction of the arrow indicate the effect of the drug injection on the corresponding element. The direction of the left arrow indicates whether amp or apo increased or decreased the frequency of the occurrence of the corresponding behavioral element relative to control saline injections. The centrally placed arrow indicates the respective differences in the duration and the right arrow in the mean duration of the behavioral elements. The two arrow lengths indicate the statistical significance of the difference (the big arrow representing a difference at the 1% level and the small arrow at the 5% level, Student's *t*-test). Only effects that were statistically significant are included here. A (—) indicates that the corresponding test was not performed, since the mean duration was not computed (the behavioral element was not recorded at the corresponding dose of amp, apo or saline).

vealed a significant drug effect with $F(1,104)=21.10$, $p<0.001$ and $F(1,104)=6.93$, $p<0.05$ respectively. The multiple range tests showed that d-amp induced more moving episodes than apo (amp-average = 196.85, apo-average = 85.71) and higher duration of moving than apo (amp-average = 278.95, apo-average = 187.4). The two-way ANOVA for the mean-duration data did not reveal any significant drug effect.

Sniffing

There was a significant dose effect for both drugs in frequency and duration of sniffing (Table 1: sniffing). The one-way ANOVA revealed a significant dose effect in mean duration of sniffing after d-amp while it did not reveal any significant dose effect of this component of sniffing after apo (Table 1: sniffing). Student's *t*-tests revealed significant effects after apo in frequency and duration of sniffing in all doses but not after 0.25 mg/kg (Table 2). On the other hand, the same tests after amp did not reveal any statistical difference in frequency and duration of sniffing except after the higher dose (6 mg/kg). The two-way ANOVA revealed a significant drug effect only in the frequency of this element, $F(1,104)=6.55$, $p<0.01$. Scheffé multiple range tests showed that apo induced less sniffing episodes than d-amp (amp-average = 181.68, apo-average = 114.64).

Grooming

There was a significant dose-effect after d-amp in all the behavioral measures while after apo there was a significant dose effect only on frequency and duration (Table 1: grooming). Student's *t*-tests revealed that at higher doses of d-amp and apo there was a decrease in grooming episodes and in the duration of them. The two-way ANOVA of frequency and duration scores with drug and dose as factors did not reveal any significant drug effect. It is interesting to note that subsequent multiple range tests of frequency scores showed that amphetamine had a tendency to induce less grooming episodes than apomorphine (amp-average = 8.88, apo-average = 15.82, $p<0.005$). The two-way ANOVA of mean-duration revealed a significant drug effect, $F(1,91)=23.1$, $p<0.001$, and the multiple range tests showed that the mean-duration of grooming after apo treatment was higher than after d-amp treatment (amp-average = 3.81, apo-average = 9.67).

Rearing

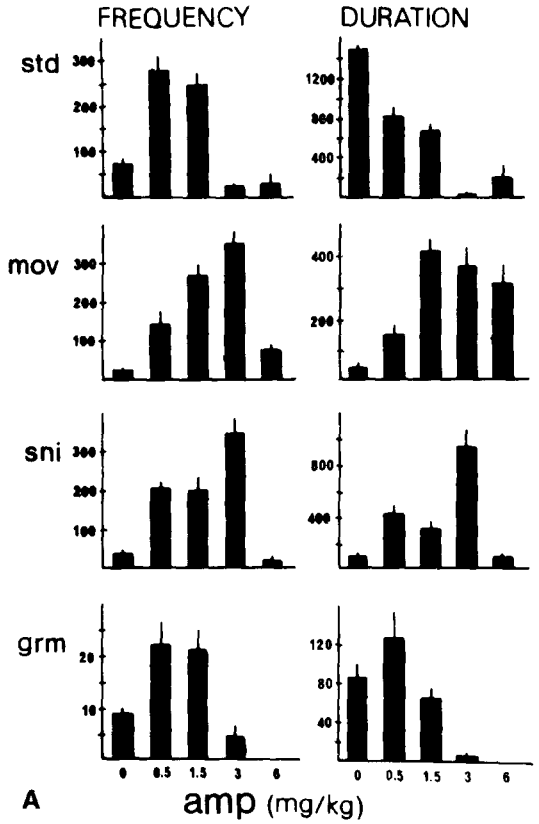
The one-way ANOVA did not reveal any significant dose effect in frequency, duration and mean-duration of rearing after apo while there was a significant dose effect in frequency and mean-duration of rearing after d-amp (Table 1: rearing). Subsequent Student's *t*-tests between d-amp and saline scores revealed a significant effect on the duration of rearing after 3 mg/kg and 6 mg/kg d-amp (Table 2). The two-way ANOVA (drug × dose) revealed a significant drug effect only on frequency and mean-duration of rearing with, $F(1,104)=13.05$, $p<0.001$, and, $F(1,93)=3.67$, $p<0.01$, respectively. The multiple range tests showed that d-amp induced more rearing episodes than apo (amp-average = 65.79, apo-average = 13.88). The same tests showed that the mean-duration of rearing after apo was higher than after amp (amp-average = 3.19, apo-average = 12.92).

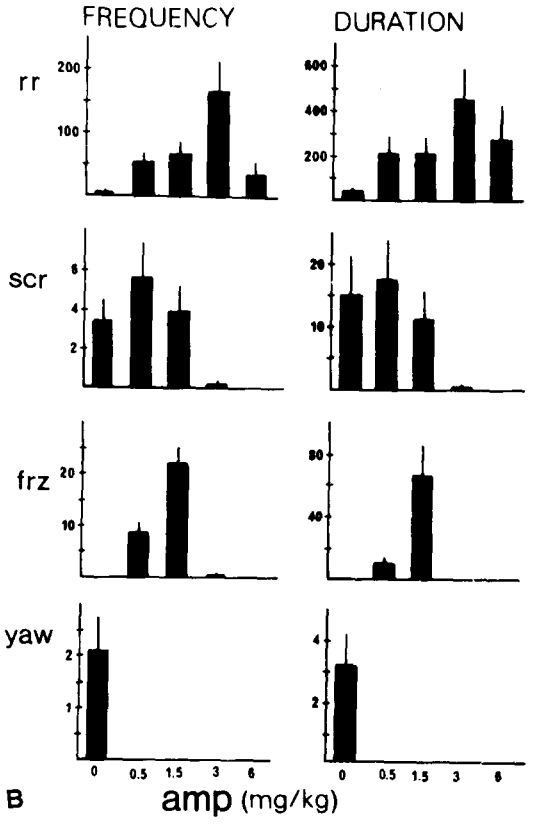
Scratching

The one-way ANOVA revealed a significant dose effect only on mean-duration of scratching after d-amp while there was a significant dose effect on frequency and duration of scratching after apo (Table 1: scratching). Student's *t*-tests showed that the higher the dose of apo the lower the frequency and duration of scratching (Table 2). The two-way ANOVA (drug × dose) revealed a significant drug effect only on frequency scores of scratching, $F(1,104)=4.06$, $p<0.05$. Multiple range tests showed that d-amp induced more scratching episodes (amp-average = 3.49, apo-average = 1.21) as well as higher duration of scratching than apo (amp-average = 11.28, apo-average = 4.88, $p<0.05$).

Freezing

In general, freezing was induced mainly by d-amp and not by apo. There was a significant dose effect on frequency and duration of this element after d-amp (Table 1: freezing). Also,





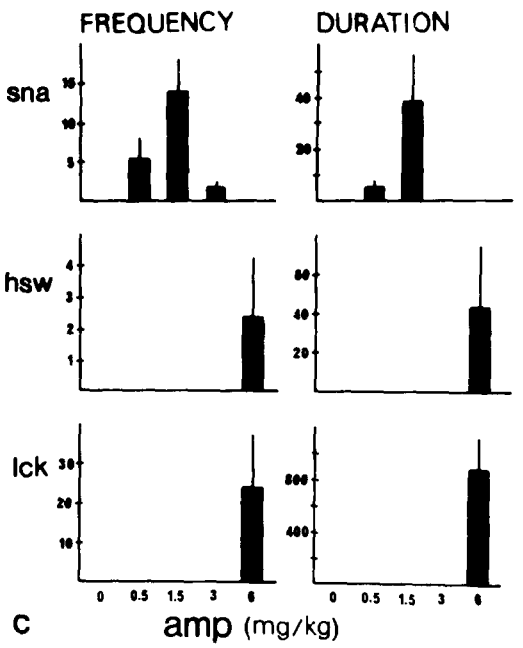


FIG. 2. (A, B, C) Effects of d-amphetamine (amp) on the frequency and duration of 11 behavioral elements. For each element the cumulative frequence scores of three 10-min recording sessions separated by 10-min intervals or the total duration in seconds of the three 10-min recording sessions are plotted arranged horizontally according to the dose of the drug (dose 0: saline).

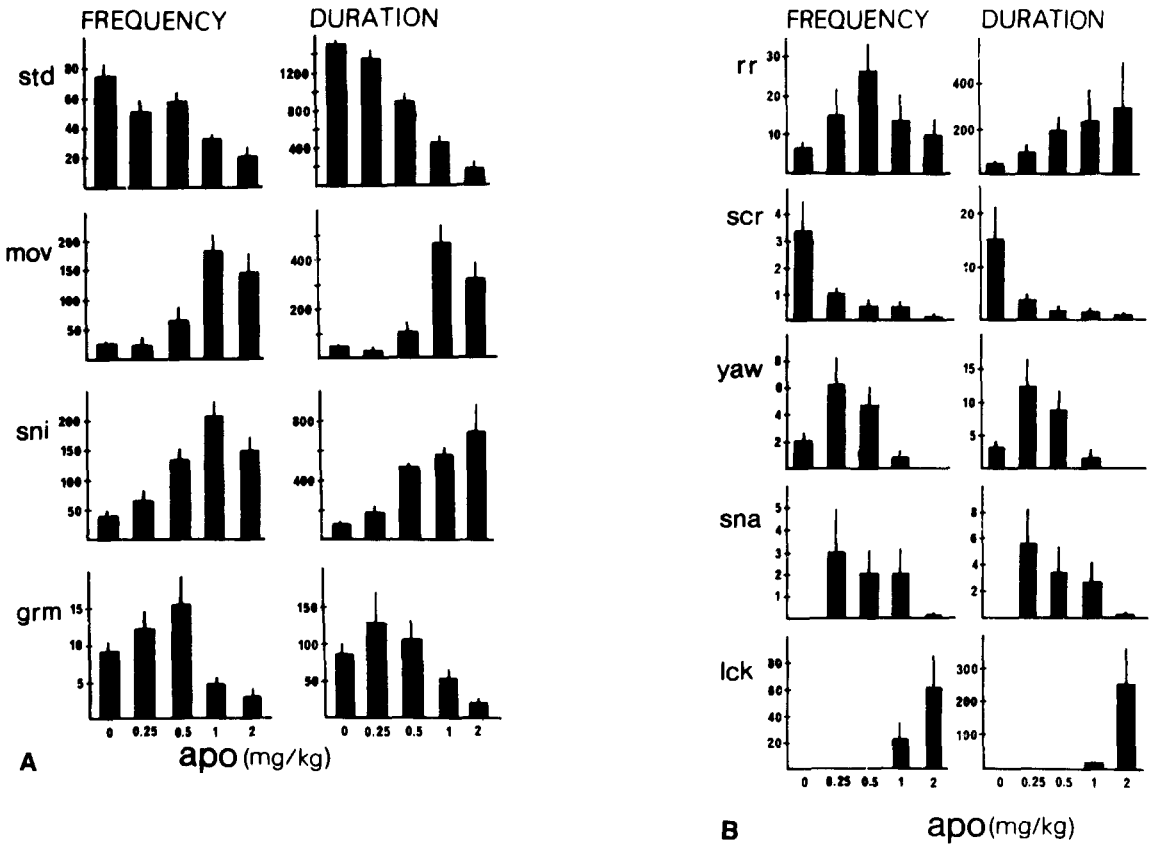


FIG. 3. (A, B) Effects of apomorphine (apo) on the frequency and duration of 9 behavioral elements. For each element the cumulative frequency scores of three 10-min recording sessions separated by 10-min intervals or the total duration in seconds of the three 10-min recording sessions are plotted arranged horizontally according to the dose of the drug (dose 0: saline).

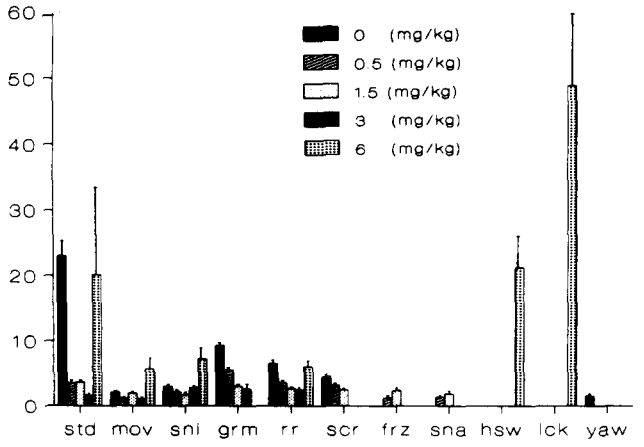


FIG. 4. Effects of four doses of d-amphetamine (amp) and saline (dose 0) on the mean duration (duration/frequency) of 11 behavioral elements: std: standing, mov: moving, sni: sniffing, grm: grooming, rr: rearing, scr: scratching, frz: freezing, sna: sniffing air, hsw: head swinging, lck: licking, yaw: yawning.

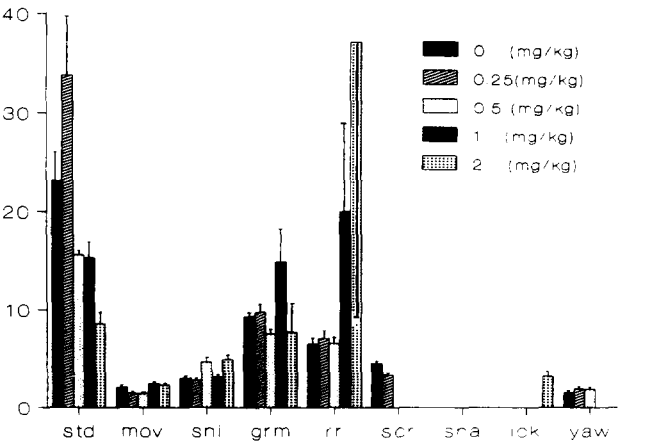


FIG. 5. Effects of four doses of apomorphine (apo) and saline (dose 0) on the mean duration (duration/frequency) of 9 behavioral elements: std: standing, mov: moving, sni: sniffing, grm: grooming, rr: rearing, scr: scratching, sna: sniffing air, lck: licking, yaw: yawning.

TABLE 3

LOADINGS OF THE OVERALL DURATION OF THE BEHAVIORAL ELEMENTS TESTED ON ALL THE SIGNIFICANT FACTORS DERIVED FROM THE FACTOR ANALYSIS AFTER D-AMPHETAMINE (AMP) OR APOMORPHINE (APO) TREATMENT

	Varimax Rotated Factor Matrix			
Duration/Factor	1	2	3	4
Percent Var	25.5%	16.6%	13.0%	10.1%
AMP				
standing	0.53	−0.47	0.43	−0.13
moving	0.18	0.64	−0.15	0.09
sniffing	−0.62	−0.35	−0.35	−0.4
grooming	−0.04	−0.38	0.66	−0.18
rearing	−0.3	0.8	−0.08	−0.13
scratching	0.09	−0.04	0.81	−0.05
sniffing air	0.7	−0.14	−0.17	−0.10
licking	−0.09	−0.09	−0.21	0.83
head swinging	−0.06	0.09	−0.01	0.76
freezing	0.7	0.04	0.13	−0.09
	Varimax Rotated Factor Matrix			
Duration/Factor	1	2	3	
Percent var	39.4	18.2	10.9	
APO				
standing	0.69	−0.02	−0.51	
moving	−0.47	−0.36	0.52	
sniffing	−0.66	−0.58	−0.01	
grooming	0.81	−0.12	−0.18	
rearing	−0.18	0.87	0.02	
scratching	0.73	−0.03	0.08	
sniffing air	−0.07	0.53	−0.45	
licking	−0.1	0.00	0.83	
yawning	0.8	−0.1	−0.24	

The percentage of the overall variation explained by the corresponding factor is indicated under the factor number.

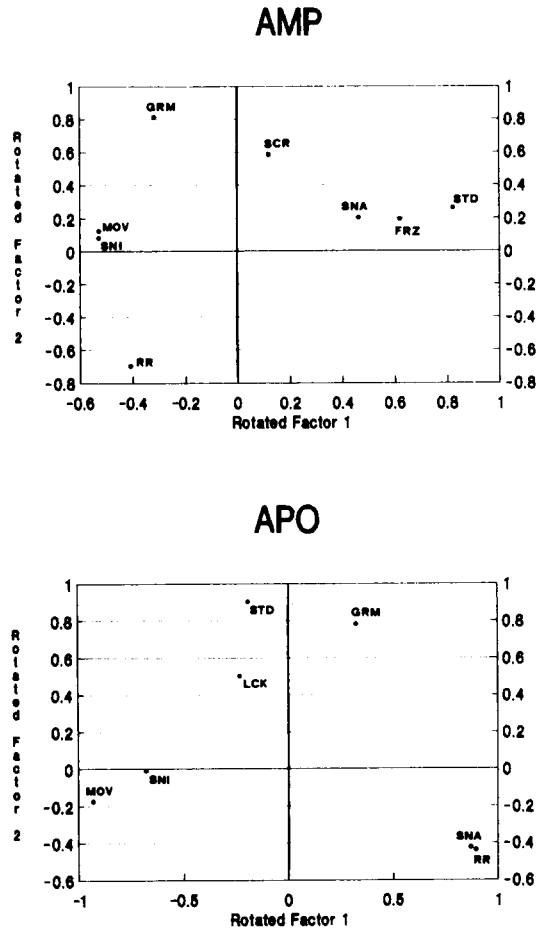


FIG. 6. Loadings of the duration of the behavioral elements tested on first two rotated factors after d-amphetamine 1.5 mg/kg (AMP) and apomorphine 1 mg/kg (APO) treatment. STD: standing, MOV: moving, SNI: sniffing, RR: rearing, SNA: sniffing air, FRZ: freezing, GRM: grooming, SCR: scratching, LCK: licking.

as shown in Fig. 1, at higher doses this behavioral element disappears.

Yawning

Yawning is a behavioral element that was induced by apo. There was a significant dose effect on frequency, duration and mean-duration of yawning after apo (Table 1: yawning). On increasing the dose of apo yawning disappears from the behavioral repertoire of the drug (Fig. 1).

Sniffing Air

The one-way ANOVA did not reveal any significant dose effect after both drugs on frequency, duration and mean duration of sniffing air. The two-way ANOVA (drug × dose) did not show any significant drug effect on frequency and duration scores of this element. On the other hand, subsequent multiple range tests on duration scores revealed that d-amp induced a higher duration of sniffing air than apo (amp-average = 16.73, apo-average = 2.21).

Head Swinging

Although head swinging was induced by d-amp at the higher dose, it did not show any statistical difference compared with saline, since the number of the rats exhibiting this behavior was too small.

Licking

d-Amp and apo induced this behavioral element at the higher doses. The one-way ANOVA revealed a significant dose effect after both drugs on frequency and duration of licking (Table 1: licking). The two-way analysis (drug × dose) showed a significant drug effect only on frequency of licking, $F(1,104) = 3.6$, $p<0.05$. Subsequent multiple range tests on frequency scores showed that apo induced more licking episodes than d-amp (amp-average = 2.07, apo-average = 16.35).

Factor Analysis

Factor analysis revealed that the overall duration data after apo were loaded on 3 components while after d-amp were loaded on 4 components. On the first factor, which explains the higher percentage variance of the data, standing, grooming, scratching, yawning and sniffing (which was in a negative correlation with the factor) were loaded after apo. The same anal-

ysis after d-amp showed that standing, sniffing air, freezing and sniffing (which was in a negative correlation with the factor) were loaded on the first factor (Table 3). As shown in Table 3, moving was loaded with rearing on the second factor after d-amp while after apo moving was loaded on a third factor with licking, although this element was also in a high correlation with the first factor. Another finding was that after d-amp only scratching and grooming were loaded on the third factor while after apo these behavioral elements were loaded on the first factor (Table 3).

In order to further analyse the behavioral elements participating in the motor activation induced by the two dopaminergic agonists, a separate factor analysis was performed on the duration data after 1.5 mg/kg of amp and 1 mg/kg of apo, since both drugs in these doses did not induce stereotyped behavior.

The factor analysis on apo data revealed that rearing and sniffing air were loaded on the first factor with a positive correlation while moving and sniffing were loaded with a negative correlation. After d-amp standing, freezing and sniffing air were loaded on the first factor with a positive correlation while moving and sniffing were loaded with a negative correlation. On the second factor, after d-amp, grooming, scratching, and rearing (which was in a negative correlation with the factor) were loaded, while after apo, standing, grooming and licking were loaded on this factor (Fig. 5).

DISCUSSION

In the present study we compared the effects of apo and d-amp, two dopaminergic agonists acting through different targets, using a multifactorial behavioral analysis in order to improve our knowledge in dopaminergic drug-induced behaviors.

The analysis of the locomotor activity as expressed by the number of photocell beam interruptions in the one-hour testing period, indicated a pronounced hyperactivity induced by both drugs versus control groups in a dose-dependent manner. These results are similar to those reported by other authors who used univariant techniques (40,45). However, the subsequent multifactorial analysis indicated different patterns of hyperactivity induced by the two dopaminergic agonists. The limitations of adopting photobeam interruption methods as a measure of behavioral response is obvious from our results, indicating that measures of behavior should include both automatic and observation criteria or continuous observation of individual rats, as suggested by some investigators (17, 25, 36, 41). Our method combines the advantages of continuous recording of behavior with the measure of every component (i.e., frequency, duration and mean-duration) of each behavioral element, according to the proposals of some authors (15, 19, 36).

d-Amp increased the frequency of every behavioral element indicating an increase in the rate of responding in agreement with Norton (33) and Lyon and Robbins (30). Apo also induced an increase in the frequency of the behavioral elements although d-amp was considerably more effective in enhancing the behavioral activation.

As shown from our results, d-amp induced more episodes of standing, moving, scratching, rearing and sniffing than apo, which induced more episodes of licking. An interesting finding was that d-amp increased the frequency of rearing (Table 2) but had no effect on the duration of this element. On the other hand, apo did not affect this element, suggesting that rearing plays a major role in shaping the behavioral response to d-amp but not to apo, in agreement with the results of Fray et al. (17).

A number of authors has suggested that d-amp enhances rearing [e.g., (7, 17, 26, 33)], but they are in contrast to the results of Bauer (2), who reported that d-amp does not influence rearing. This discrepancy, taken together with our results, could be explained by the hypothesis that the well known increase in rearing after d-amp does not represent an increase of its duration, but an increased number of rearing episodes during the observation period.

Another difference between d-amp and apo concerns the freezing behavior. d-Amp induced this element in low doses and mainly in the dose 1.5 mg/kg, while apo did not induce freezing at all. Recording the sequence of behavior after d-amp it was observed that freezing underlined the behavioral profile since the rat behavior was often interrupted by unpredictable freezing. This element as an immobility reaction is an expression of fear and anxiety, and its duration is reduced as the age increases (22).

On the other hand, apo enhances the frequency and the duration of yawning, an element that is not induced by d-amp and reflects the direct stimulation of autoreceptors. It is exhibited after low doses of apo in combination with decreased locomotor activity, in agreement with Strombom (44) and Mogilnicka Klimek (32).

The multivariate assessment of behavior by factor analysis revealed a number of differences in the behavioral profiles elicited by d-amp and apo. In general, four factors explained 67% of variance in the case of d-amp, while three factors explained the same percentage in the case of apo. This finding indicates that not only were there elements that were not participating in the response to apo, such as rearing or freezing, but whole categories of elements that participated in the d-amp-induced behavior were not present in the apo-induced behavioral response. This is in agreement with the general view that apo induces a restricted range of responses compared to d-amp (20). Another striking finding is that the stereotyped elements were loaded on the last factor for both drugs and with the same variance (approx. 10%) (Table 3). Since stereotyped behavior was elicited by higher doses of both drugs, this finding indicates that at these doses the behavioral pattern for both drugs is similar and is characterized by repetitive movements of great duration that reflect the stereotyped nature of these behavioral elements.

In order to better characterize the locomotor profiles elicited by the two drugs without interfering with stereotyped responses, we selected the two low doses (1 mg/kg apo and 1.5 mg/kg d-amp) for a separate factor analysis.

These two analyses revealed that while factor 1 explains 46% of the total variance in the case of apo, it explains only 30% in the case of d-amp. It is interesting to note that on factor 1, after apo, four behavioral elements were loaded while after d-amp, five behavioral elements were loaded. This finding indicates that the behavioral profile of apo is mainly characterized by moving and sniffing interrupted by rearing and sniffing air, while the amp-induced profile is mainly characterized by sniffing and moving, interrupted by standing, freezing, and sniffing air.

From our data, however, it seems that both drugs increased locomotor activity, while inducing stereotyped behavior in high doses, influencing probably the dopaminergic function on two separate neural systems. It has been demonstrated that the locomotor effects of the low doses of d-amp and apo were due to the activation of mesolimbic DA system, while the stereotyped behavior after high doses was induced by the preferential activation of the nigrostriatal DA system (27–29).

However, although apo and d-amp have a common dose-dependent activation on the neural dopaminergic systems, their behavioral pattern is based on a different structure.

In the case of apo, where moving is loaded on all factors (Fig. 6), this pattern of behavior is organized mainly around

moving, e.g., the rat is performing every other element interrupting a continuous moving around the cage. On the contrary, the pattern of d-amp is organized around standing which was loaded in all the factors, indicating that this element influences the duration of all the behavioral elements.

Furthermore, our results could support the hypothesis that d-amp, increasing the frequency and decreasing the mean duration of an act, except in the higher doses where intensive over time acts exist, enhanced rate of transition from one element to the other, indicating a response switching and repetition mainly in the higher doses. The behavioral profile, therefore, after d-amp treatment, could be characterized by the words "unpredictable pause" and "unpredictable respond" of the occurring response based on a "probability dependence" and not only on "rate dependence" or "dose dependence," as some authors have suggested (13,37).

On the other hand, in the case of apo a less complex scheme could be proposed. The profile seems to be based on "predictable respond" and a "predictable pause" of the occurring response, in a perseverative manner more effective than amp and with a restricted range of behavioral elements that are mainly influenced by dose changes.

Our data shows that apo and d-amp elicited marked differences in behavioral profile. The mechanism of these differences may be attributed to the different modes of actions of these two dopaminergic drugs. It is worth noting that d-amp acts by increasing release and inhibiting reuptake of dopamine, and it has been suggested that its behavioral effects appear to be influenced by a sensory feed-back (38). In contrast, apo acts via dopaminergic receptors where the low doses stimulate the autoreceptors and this results in behavioral inhibition (6,11), while the higher doses stimulate the postsynaptic receptors reversing the behavioral inhibition thus suggesting that its behavioral output is not directed by sensory stimuli (25). In addition, d-amp in low doses induces a dopamine release in striatal and limbic areas without affecting the firing rate of the mesencephalic dopaminergic neurons (4,46). As a consequence, it does not alter the behavioral profile of the animal, preserving the normal responses though at an enhanced level. On the contrary, at high doses, d-amp inhibits the firing rate of the mesencephalic dopaminergic neurons (4,46) and the dopamine release within the terminal fields is not coupled to neuronal firing. Thus the activation of striatal and limbic dopaminergic receptors is nonspecific and independent of information flow through the mesostriatal and mesolimbic systems, resulting in a restricted and perseverative behavioral profile. This profile is similar to that induced by apo, since this drug acting directly on postsynaptic dopaminergic receptors induces also an activation which is not coupled to mesostriatal and mesolimbic impulse flow.

ACKNOWLEDGEMENTS

The authors thank Dr. T. Hyphantis, M.D., Medical School, Department of Psychiatry, University of Ioannina and V. Raissis, Research Fellow, School of Letters, Department of Philosophy, University of Ioannina, for critical reviews of this manuscript and for many helpful and informative discussions.

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