Pharmacology Biochemistry & Behavior, Vol. 21, pp. 191–195, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Effect of d-Amphetamine and Diazepam on the Greeting Behavior of Rhesus Monkeys (*Macaca mulatta*)

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Received 6 July 1983

THIERRY, B. H., C. L. MILHAUD AND M. J. KLEIN. *Effect of d-amphetamine and diazepam on the greeting behavior of rhesus monkeys* (Macaca mulatta). PHARMACOL BIOCHEM BEHAV 21(2) 191–195, 1984.—The greeting behavior of rhesus monkeys *(Macaca mulatta)* was evaluated in a pharmacological test. It was observed in pairs of juvenile subjects reunited after a separation of two days. The observational measures were the duration of greeting behavior (social grooming, social play, and huddling) and the frequency of presentations, mounts and solicitations. d-Amphetamine (0.2 and 0.1 mg·kg^{-1}) was found to shorten the duration of greeting behavior and increase the frequency of presentations and mounts. Diazepam (1 and 0.5 mg·kg^{-1}) was found to prolong the duration of greeting behavior.

Psychopharmacology Social behavior Greeting behavior d-Amphetamine Diazepam Rhesus macaque

MANY studies have been conducted on nonhuman primates in order to assess the action of psychotropic drugs on their social behavior [17,29]. These animals offer a very interesting model due to their phylogenetic closeness to man and to their tendency to live in permanent and highly structured social groups. However, the complexity of their social structure makes it difficult to develop behavioral tests which meet Janssen's criteria [13], i.e., reliability, sensitivity, specificity, simple and rational experimental procedure. This accounts for the many different methodologies applied by various authors. Until now, most investigations carried out have focused on the action of psychotropic drugs on the overall behavior of these animals without any real standardization. Some authors studied a particular social relationship such as that between mother and offspring [14, 21, 30] or dominance-submission during food competition [3, 7, 11, 18]. The great diversity of species, environmental conditions and animal group patterns (pairs, groups of a few subjects, troops) and the changes in observational measures may explain the apparently contradictory results often obtained by different authors. As an example, some authors observed that haloperidol antagonizes the effects of d-amphetamine on social behavior [10, 25, 33] whereas others have drawn opposite conclusions [22, 28, 30, 34].

In order to standardize our experimental conditions, we tried to work in a social situation which could meet Janssen's criteria for pharmacological testing [13]. A good instance of this type of situation is the greeting behavior of rhesus macaques *(Macaca mulatta)*. Several authors [4, 8, 9, 20] have demonstrated that when juvenile individuals are separated for periods of time ranging from two days to two weeks, their reunion is characterized by extensive social interactions. These interactions are relatively simple: they include social grooming, mounts, embrace and huddling. However, this behavior is only exhibited if the reuniting subjects have been living together for several months prior to separation.

In a preliminary investigation [36], greeting behavior was shown to be reliable. The aim of the present study was to assess the value of this behavior in a pharmacological test. Two psychotropic drugs inducing well-known behavioral effects were tested at commonly used doses: a psychostimulant, d-amphetamine [3, 16, 30] and an antianxiety agent, diazepam [7, 18, 23].

METHOD

Subjects

The experiment was conducted on 14 rhesus macaques *(Macaca mulatta)* between 2½ and 4 years old which were born in the laboratory. Subjects were kept in 60×50×70 cm cages for more than six months. They included three pairs of males (A-B, C-D, E-F), three pairs of females (I-J, K-L, M-N) and one mixed pair (G-H). They were fed twice a day and given water ad lib. Lighting was controlled to provide a 7:00–19:00 day/night cycle.

Procedure

The experimental protocol consisted of two phases: a separation and isolation phase which lasted two days during which two subjects of a pair were separated from each other and deprived of visual contact with other animals, and a one-hour reunion phase during which both subjects of the

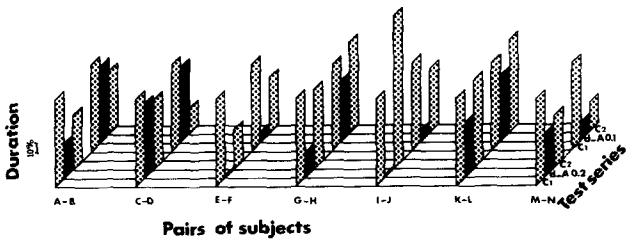

FIG. 1. Effect of d-amphetamine on the duration of greeting behavior. The duration of greeting behavior consists of the sum of duration of social grooming, social play and huddling for one hour of observation. For each dose, subject pairs were submitted to a pre-treatment control test (series C_1: dotted bars) which was taken as 100% reference baseline, followed by a treatment test (series d-A 0.2 mg·kg^{-1} and d-A 0.1 mg·kg^{-1}: solid bars) and a post-treatment control test (series C_2: dotted bars).

separated pair were reunited in an observation room. This observation room was a 2×2 m, air conditioned, partially sound-proof enclosure. Subjects were reunited in a 60×50×70 cm cage, placed at the center of the room. A one-way mirror placed above the subjects permitted easy observation of the subjects, which could not see their reflection.

Before the experiment, subjects had experienced multiple control separation-reunion events [36]. In the experiment, for each type of drug treatment, three consecutive tests were performed on each pair at three-week intervals: (1) a pre-treatment control test (C_1); (2) a test where both subjects of the same pair were treated; (3) a post-treatment control test (C_2). Four different drug treatments were used: 0.1 and 0.2 mg·kg^{-1} d-amphetamine (solution of 1 mg·ml^{-1} administered IM one half-hour before observation), and 0.5 and 1 mg·kg^{-1} diazepam (solution of 5 mg−ml^{-1} administered IM fifteen minutes before observation). The four test series were performed in the following order: d-A 0.2 mg·kg^{-1}; dzp 1 mg·kg^{-1}; dzp 0.5 mg·kg^{-1}; d-A 0.1 mg·kg^{-1}. In control tests, subjects were injected with saline.

Behavioral Measures

The observer recorded the behavior of both subjects of the pair, with no distinction between individuals. Some behaviors were recorded in terms of duration, others in terms of frequency, using an Apple II microprocessor.

For behaviors measured in terms of duration the time unit was the second. These behaviors included: social grooming, e.g., investigation of the partner's body using hands or mouth; social play, e.g., rough and tumble play; and huddling where reunited subjects hugged each other, either ventro-ventrally or dorso-ventrally. The sum of the duration of these behaviors was considered as the duration of "greeting behavior."

For behaviors measured in frequency, behaviors repeated at intervals of less than five seconds were recorded as a single event. These included mounts: the subject gripped and mounted the rear part of its partner; presentation: the subject extended its rear part toward its partner while raising its tail or turning it to the side; solicitation: several behaviors which, in the test situation, were designated to invite social grooming; play or huddling: the subject scratched or groomed itself, or lay on the ground, or pulled its partner's arm or leg, or positioned its partner's hindlegs to try and mount it.

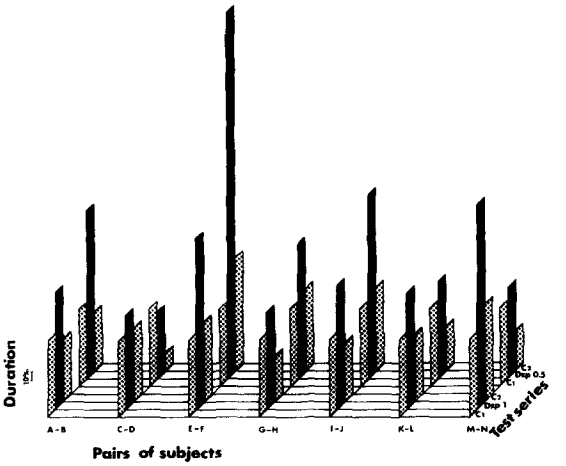

FIG. 2. Effect of diazepam on the duration of greeting behavior. For each dose, subject pairs were submitted to a pre-treatment control test (series C_1: dotted bars) which was taken as 100% reference baseline, followed by a treatment test (series Dzp 1 mg·kg^{-1} and Dzp 0.5 mg·kg^{-1}: solid bars) and a post-treatment control test (series C_2: dotted bars).

Statistical Analysis

Comparisons between series of tests used one-way repeated-measures ANOVA with controls and treatments as the repeated measures [37].

RESULTS

Administration of d-amphetamine reduced the duration of greeting behavior, $F(1,6)=12.8$, $p<0.05$, but without a dose effect, $F(1,6)=0.03$, NS (Fig. 1). There was an increase in the frequency of presentations, $F(1,6)=35.7$, $p<0.001$, and mounts, $F(1,6)=6.3$, $p<0.05$; the increase in the frequency of presentations was more pronounced at 0.2 than 0.1 mg·kg^{-1}, $F(1,6)=29.2$, $p<0.01$; there was no dose effect for mounts, $F(1,6)=1.9$, NS. No change was induced by d-amphetamine in frequency of solicitations, $F(1,6)=0.6$, NS (Table 1).

Administration of diazepam prolonged greeting behavior, $F(1,6)=40.4$, $p<0.001$ (Fig. 2) but without dose effect, $F(1,6)=1.9$, NS. No changes were induced by diazepam in frequency of presentations, $F(1,6)=3.3$, NS, mounts, $F(1,6)=5.2$, NS, or solicitations, $F(1,66)=2.6$, NS (Table 1).

As indicated earlier, subjects served in four experimental tests. In order to assess the effect of drug treatment on the subsequent behavior of subjects, series of pre-treatment tests were compared with series of post-treatment tests. Duration of greeting behavior was found to be longer in series C_1 than in series C_2, $F(1,6)=6.1$, $p<0.05$, thus an order effect might have occurred regarding the duration parameter. However, no statistically significant differences were found in frequency measures (presentations: $F(1,6)=0.3$; mounts: $F(1,6)=0.6$; solicitations: $F(1,6)=0.6$).

DISCUSSION

With respect to d-amphetamine, the results are in agreement with most published data about its effects on the social behavior of primates. As a general rule, at doses of 0.05–4.00 mg·kg^{-1} (acute, subchronic or chronic doses), the main effect of d-amphetamine is to isolate the individual through decreased social interactions with conspecifics [6, 10, 15, 16,

TABLE 1
FREQUENCY OF INTERACTIONS

		Pairs of Subjects						
		A-B	C-D	E-F	G-H	I-J	K-L	M-N
Solicitations	C1	112	30	16	10	37	36	18
	d-A 0.2	37	21	9	20	20	13	10
	C2	41	29	9	14	7	24	17
	C1	31	24	21	20	28	15	11
	Dzp 1	61	37	33	34	42	8	28
	C2	42	30	6	36	34	9	13
	C1	43	36	16	17	16	12	10
	Dzp 0.5	29	42	8	29	56	9	25
	C2	63	36	4	34	25	5	12
	C1	37	45	6	12	30	13	12
	d-A 0.1	46	41	3	44	32	9	7
	C2	44	38	8	28	17	12	18
Presentations	C1	15	8	11	17	35	5	3
	d-A 0.2	54	33	4	51	101	9	10
	C2	9	10	0	8	22	2	7
	C1	14	6	2	15	31	2	5
	Dzp 1	12	10	5	5	25	0	1
	C2	6	11	0	16	40	2	2
	C1	6	9	2	12	36	4	0
	Dzp 0.5	8	8	4	5	36	0	1
	C2	11	12	1	8	18	2	1
	C1	8	7	2	2	15	5	2
	d-A 0.1	26	17	2	17	20	4	0
	C2	6	11	3	9	24	1	1
Mounts	C1	9	14	2	1	11	0	1
	d-A 0.2	40	20	1	5	5	0	0
	C2	9	11	1	3	5	0	3
	C1	23	7	0	3	9	0	3
	Dzp 1	15	13	4	8	12	0	0
	C2	14	8	1	9	8	0	0
	C1	23	9	1	2	11	0	0
	Dzp 0.5	22	24	2	2	13	0	1
	C2	13	12	2	2	7	0	3
	C1	10	11	4	1	5	0	2
	d-A 0.2	18	21	4	0	6	0	0
	C2	7	9	3	0	7	0	0

Values represent the frequency of solicitations, presentations and mounts for a one-hour observation period. Each one of the seven pairs underwent a series of four tests where both subjects were treated (d-A 0.2 $mg \cdot kg^{-1}$; Dzp 1 $mg \cdot kg^{-1}$; Dzp 0.5 $mg \cdot kg^{-1}$; d-A 0.1 $mg \cdot kg^{-1}$). Each one of these tests was preceded by a pre-treatment control test (C1) and followed by a post-treatment control test (C2).

19, 21, 22, 27, 28, 30, 31, 33, 34]. Some authors report contradictory results [3, 5, 12, 25, 26, 32], they mostly used relatively small doses and their results were probably greatly influenced by social factors. It has been demonstrated that the effect of amphetamine on the behavior of primates varies as a function of the social context in which the subjects happen to be: the size of the group, the presence of preferential partners and the status of the individual in the hierarchy seem to be of special significance [3, 5, 12, 18, 21, 33].

With respect to diazepam, the present data corroborate results generally obtained by authors who have studied its effects on the social behavior of primates. Similar to other benzodiazepines, diazepam, at doses of 0.1–3 $mg \cdot kg^{-1}$ (acute doses) enhances affiliative behaviors and reduces agonistic interactions [1, 2, 7, 23, 24]. Here too, the effect of the psychotropic drug can vary as a function of dominance-submission relationships which exist between subjects [7, 11, 18].

As a pharmacological test, greeting behavior has been shown to be both sensitive and straightforward, its use meets the major criteria defined by Janssen [13] and exposes the classical effects of d-amphetamine and diazepam on the social behavior of primates. However, subjects should not be submitted to too many tests to avoid possible habituation to the testing situation and, thus, non specific effects of drug treatments. In addition, as for other tests of social behavior,

caution must be used in interpretation of results. All experimental situations are, by definition, biased when they concern primate social behavior. Most primates normally live in groups where each individual establishes specific relationships with each one of the group members over a number of years. In the case of greeting behavior, test subjects were paired and placed in an artificially created "crisis situation." Although these conditions are interesting from a strictly pharmacological standpoint, difficulties of interpretation may arise as to what the exact effect of psychotropic agents on the behavior may be. Indeed, the meaning of signals exchanged by individuals varies depending on the context in which they occur [35]. For example, the frequency of presentation increased in subjects treated with amphetamine. Other authors [3,33] have already shown this fact and concluded that amphetamine either stimulates sexual behavior, or enhances submissive behaviors. However, presentation may have different meanings: depending on the context, it can be a sexual invitation, a sign of submission, an appeasement display, an alliance request, a grooming solicitation, a play behavior, etc. In the present study, amphetamine also induced an increase in the frequency of mounts: this may mark a real increase in sexual behavior, but another explanation is that it is a direct consequence of the heightened frequency of presentations.

On the whole, the qualities of the greeting test clearly demonstrate its utility as a complementary test in the pharmacology of primate social behavior.

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