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Time Sampling Observation Procedure for Studying Drug Effects: Interaction Between *d*-Amphetamine and Selective Dopamine Receptor Antagonists in the Rat

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PETRY, N., L. FURMIDGE, Z.-Y. TONG, C. MARTIN AND D. CLARK. *Time sampling observation procedure for studying drug effects: Interaction between d-amphetamine and selective dopamine receptor antagonists in the rat.* PHARMACOL BIOCHEM BEHAV **44**(1) 167–180, 1993.—A rapid time sampling observation procedure combined with two forms of automatic activity assessment is described. The methods are illustrated by examination of the behavioral effects of *d*-amphetamine, administered to rats either alone or in combination with antagonists selective for D_1 or D_2 dopamine (DA) receptors. Low doses of *d*-amphetamine (0.5–4.0 mg/kg) increased photocell counts, rearing, ambulation, and various forms of sniffing. Similar effects were observed in the first 30 min following administration of 8.0 mg/kg *d*-amphetamine. However, animals exhibited licking, intense sniffing down, and repetitive head and limb movements in the following 30 min; minimal ambulation, rearing, and sniffing up were observed. The "traditional" total photocell count measure did not differentiate between these time-dependent changes in locomotion. On the other hand, latch counts—where subjects had to move between the beams to register a count—adequately demonstrated this change in locomotion. The selective D_1 receptor antagonist SCH23390 and selective D_2 antagonist raclopride dose dependently inhibited the behavioral changes produced by 1.5 mg/kg *d*-amphetamine. Higher doses of SCH23390, but not raclopride, produced a behavioral pattern indistinguishable from that observed in control sessions. Both DA antagonists equipotently blocked the intense sniffing down and repetitive head/body movements produced by 8.0 mg/kg *d*-amphetamine. A narrow intermediate range of doses of SCH23390 reduced the incidences of these behaviors and produced levels of locomotion and sniffing straight that were significantly higher than those observed in control session. This form of behavioral activation was not observed with raclopride. Therefore, this observation procedure revealed subtle differences between the inhibitory effects of SCH23390 and raclopride on *d*-amphetamine-induced behavioral changes.

d-Amphetamine Locomotor activity Stereotypy Rat Dopamine antagonists D_1 and D_2 receptors Activity assessment Behavioral observation

SYSTEMIC injections of *d*-amphetamine have long been known to induce a variety of behavioral changes that depend upon the dose administered. In the rat, low doses of the drug enhance locomotor activity, rearing, and sniffing. Higher doses of the drug produce highly repetitive and invariant (stereotyped) patterns of behavior that can include sniffing of the floor and oral activities such as licking and gnawing (13,28,37). The alterations in behavioral response to increasing dose of *d*-amphetamine can be accounted for by a response incompatibility hypothesis detailed by Lyon and Robbins (22), who proposed that *d*-amphetamine increases the response rate of all behaviors, but as the dose is increased the range of motor acts becomes more restricted due to a form of behavioral competition that only allows those responses with short discrete components to be elicited.

The stereotypy induced by *d*-amphetamine, and other dopamine (DA) receptor agonists, is in general assessed by rating scales (8,9,12). The disadvantages of such an approach have been emphasized by various investigators (13,19,29,31). An implicit assumption in the use of these scales is that different

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responses are ordered along a specific continuum of intensity, irrespective of the drug or experimental conditions. However, a variety of experimental findings indicate that this assumption is unwarranted (7,21,33). Use of rating scales, moreover, suffers from problems of subjectivity and limits the statistical tests that can be applied to the data (31). Most importantly, these scales can result in loss of information of the specific form of behavioral response. This problem naturally impedes efforts to determine the neural mechanisms underlying distinct behavioral changes produced by DA agonists and the manner in which drugs can influence hyperdopaminergic states.

In light of these problems, and those associated with sole use of automatic assessment of locomotor activity (13,31), various investigators have adopted a procedure that combines automatic activity measurement with an observation technique involving description and scoring of distinct behavioral categories (2,13,25,32). We recently developed a computer-supported rapid time sampling observation technique that allows us to make precise statements about drug effects on specific behavioral categories. We combined this method with two forms of automatic activity measurement. In many experimental situations, the interruption of photocell beams (often two) is used to assess changes in locomotor activity. However, when all photocell counts are recorded the locomotion measure is likely confounded by other types of activity such as head movements and grooming. We therefore adopted a similar procedure to that used by Sahgal and colleagues (34) to assess general movement around the cage. Photocell cell beams at the front and the rear of the cage had to be interrupted in strict sequence (one followed by the other) for a latch, or locomotion, score to be registered. A computer continuously monitored both latch and total photocell counts.

In the present article, we provide an illustration of the use of these techniques to determine the behavioral changes produced by various doses of *d*-amphetamine. In addition, we compared the ability of the selective D_1 receptor antagonist SCH23390 (14) and selective D_2 antagonist raclopride (16) to inhibit the behavioral changes produced by low- and high-dose *d*-amphetamine. Despite acting at different receptors, SCH23390 has been reported to exert similar antagonistic effects to those observed with D_2 antagonists on stimulant-induced unconditioned behavior in normal rats (1,23). This effect is thought to occur because SCH23390 blocks D_1 receptor-mediated enabling of the expression of D_2 receptor behaviors [see (5) for review]. However, these studies do not exclude the possibility that subtle differences exist between the inhibitory effects of selective D_1 and D_2 antagonists.

METHOD

Subjects

Sprague-Dawley albino rats (Harlan Olac Ltd., Bicester, UK) weighing 250–360 g at the start of the studies were used. They were housed in pairs in a room maintained at a constant temperature (21–23°C). Lights were on between 0700–0900 h, and free access to food and water was provided. At least 1 week separated the arrival of animals in the laboratory and the start of the studies.

Apparatus

Experiments were conducted in eight identical clear Perspex boxes measuring 35 × 35 × 30 cm and containing a metal grid floor (1 cm^2 mesh). Screens were placed between the individual boxes to prevent visual distraction.

Locomotor activity was measured by means of two infrared photocells and their associated detectors. They were positioned on the sides of the box, 18 cm apart and 5.5 cm above the floor. The detectors, which were set to ignore beam breaks occurring within 0.5 s of the previous interruption, were linked to an Amstrad PC1640 via a PC-62 optically isolated input card (Amplicon Liveline Ltd., Brighton, UK). Activity counts were recorded by software written in TopSpeed Modula-2 (see the Procedures section for further details).

Procedures

For the *d*-amphetamine dose-response study, 48 rats were randomly assigned to one of six dose conditions. Animals in this experiment were used only once; they were placed singly in the activity boxes and allowed a 2-h habituation period. After this time, they were injected with *d*-amphetamine or saline and immediately returned to the activity box. A 1-h period of locomotor activity monitoring and behavioral observation commenced immediately after the last rat was injected. Experimental sessions were carried out in the afternoon.

The various behavioral activities exhibited by rats were recorded by a rapid time sampling procedure. These behaviors are described in Table 1. Animals were observed for a period of 25 s every 5 min. A tone was generated every 5 s through an earpiece, at which time the observer would record any behavior, or combination of behaviors, being exhibited by an individual rat. This sampling technique was repeated on five occasions for the same animal. The same procedure was then carried out for the remaining seven rats, after which time the observer rested for a period of 100 s. The overall process was repeated a further 11 times, providing a total of 60 data points for each rat. The observer was unaware of the particular dose

TABLE 1

DEFINITION OF THE BEHAVIORAL CATEGORIES SAMPLED DURING OBSERVATION PERIODS

Ambulation	Movement of at least three legs in forward direction
Rear	Supported by hind limbs, forepaws raised off floor and may be placed against the wall
Sniff up	Sniffing, head and snout raised
Sniff down	Sniffing, head and snout lowered
Sniff straight	Sniffing, head horizontal
Repetitive movement	Repeated small movements of head and limbs with animal remaining in the same place
Lick	Protrusion of tongue against grid or wall
Gnaw	Gnawing of grid, teeth visible
Eating	Holding and nibbling feces
Oral movement	Chewing motion, in absence of eating or gnawing
Groom	Grooming of head or body
Standing still*	No detectable movement other than occasional sniff; animal standing still
Lying inactive*	No detectable movement other than occasional sniff; animal lying down

The behavior(s) occurring at the time of the tone were noted.

*These scores were combined to form an *inactive* score because they did not provide any additional information about the profile of the drugs.

of drug administered. Interobserver reliability using this procedure is high (Pearson's $r = 0.9, p < 0.00001$).

A different group of 32 rats was used for the DA antagonist studies. They were allocated a particular activity box and habituated for 1 h on 3 consecutive days prior to the experiment. On the days preceeding each drug treatment, further 30-min habituation sessions were carried out. Drug sessions were between 1300 and 1700 h on Tuesdays and Fridays. Rats were arbitrarily divided into groups of eight for the following studies: SCH23390 in combination with 1.5 mg/kg *d*-amphetamine; SCH23390 and 8.0 mg/kg *d*-amphetamine; raclopride and 1.5 mg/kg *d*-amphetamine; raclopride and 8.0 mg/kg *d*-amphetamine. Each animal was administered several doses of the antagonist, including the drug vehicle, and also received the vehicle-saline condition. The particular dose order for each rat was determined randomly.

Raclopride and SCH23390 (and their vehicle) were administered 45 and 30 min, respectively, before the experimental session. *d*-Amphetamine was injected 25 min prior to the animal being placed in the activity boxes and the start of the 30-min observation period. Behavior was monitored in an identical fashion to that described above except only 30 samples were collected for each rat. Again, the observer was blind to the particular drug treatment.

Computer Program

The behavioral frequencies were scored using the keyboard of an Amstrad PC1640, which provided input software written in Top Speed Modula-2 (Jensen & Partners UK Ltd.) in the laboratory. Our software permits scoring of either individual behaviors or those occurring in combination. The observer presses the key(s) referring to the behavior(s) followed by the <return> key to indicate that the particular sample is complete. After five samples, the computer produces a tone to indicate to the experimenter that the next rat is to be observed. Samples for the eight boxes are shown on screen and the relevant window is cleared each time the sampling for the last rat is completed. The software also permits correction of errors. At the end of the session, a printout is provided of the scores for all the behavioral categories, including any combinations of behaviors, during each 5-min interval.

The software also monitors the photocells throughout the experimental session via the PC-62 optically isolated input card. Monitoring involves counting the number of interruptions of the two photocell beams, thus providing details of the total photocell counts (gross movement) and latch (locomotion) counts. To obtain a latch count, the photocell beams need to be interrupted in strict sequence, that is, the rat must move through the front beam and then the rear beam, or vice versa. The total photocell and latch counts, and the scores for each 5-min period, are printed at the end of session. Latch counts can also be monitored on the screen during the study.

The behavioral samples and activity scores for each rat can readily be transferred to a commercial spreadsheet and/or to the SAS statistics package for analysis.

Drugs

The following drugs were dissolved in saline and administered IP in a volume of 1 ml/kg body weight: *d*-amphetamine sulphate (Sigma Chemical Co. Ltd., Poole, UK), SCH23390 (Schering Plough, Kenilworth, NJ), and raclopride (Astra Alab AB, Södertälje, Sweden).

Statistics

For the dose-response experiment, the frequency of occurrence of the individual behaviors and the photocell data were analyzed using a two-way analysis of variance (ANOVA; SAS Institute, Cary, NC) with dose as the between-group factor and time (15-min periods) as the within-group factor. Duncan's posthoc comparison of means were used after the relevant simple main effects tests. The DA antagonist experiments were analyzed by a one-way ANOVA followed by Duncan's test. In both forms of study, the data were square root transformed [$y = \sqrt{(x + 0.5)}$] prior to analysis to stabilize variances and decrease skewness. Relevant correlations were carried out using Pearson's product-moment correlation analysis.

RESULTS

d-Amphetamine Dose-Response

The predominant behavioral activities produced by *d*-amphetamine included locomotion, rearing, sniffing, and, at higher doses of drug, intense repetitive movement and licking. Sniffing took three forms: upward sniffing with the head raised; downward sniffing, which focused on the cage floor; or sniffing with the head held horizontal. The frequency of occurrence of these behaviors and the number of photocell (gross movement) and latch (locomotion) counts over the 1-h observation period are illustrated in Fig. 1. In addition, we have shown the effects of *d*-amphetamine on selected behaviors during 15-min periods of the experimental session (see Fig. 2).

Total photocell counts (gross movement). The two-way ANOVA only revealed a significant effect of dose, $F(5, 42) = 7.06, p < 0.0001$. All doses of *d*-amphetamine increased total photocell counts, with the two highest doses producing a more marked effect than the two lowest (Fig. 1).

Latch counts (locomotion). The two-way ANOVA revealed a significant dose × time interaction, $F(15, 126) = 7.11, p < 0.0001$. The posthoc comparison of means test on the simple main effects revealed that 2.0–8.0 mg/kg *d*-amphetamine increased the number of latch counts during the first 15-min period (Fig. 2). The highest dose of drug produced a level of activity significantly higher than that observed with the other doses. However, all doses produced an equivalent increase in latch counts during the second period. A consistent pattern of effects was observed during the last two periods; all but the highest dose of drug increased latch counts. In fact, activity was almost absent after 8.0 mg/kg *d*-amphetamine during the last 15 min of the session. Animals remained in a restricted location and displayed highly focused licking and sniffing down during the last half of the session.

Pearson's product-moment analyses revealed that the latch and total photocell counts were only significantly correlated after 1.0–4.0 mg/kg *d*-amphetamine ($r = 0.84$–$0.96, p < 0.003$–0.0001).

Ambulation. The incidence of ambulation assessed by the time sampling procedure was fairly low. Ambulation often took the form of rapid and short-lasting movements from one location to another. Although the two-way ANOVA revealed a significant effect of dose, $F(5, 42) = 3.23, p < 0.02$, the increases were not dose dependent. All but the 1.0-mg/kg dose of *d*-amphetamine increased ambulation (Fig. 1). The incidence of ambulation only correlated with the automated locomotion measure after the lowest dose ($r = 0.92, p < 0.0005$) and two highest doses of *d*-amphetamine ($r = 0.81, p < 0.02$, and $r = 0.85, p < 0.007$, respectively).

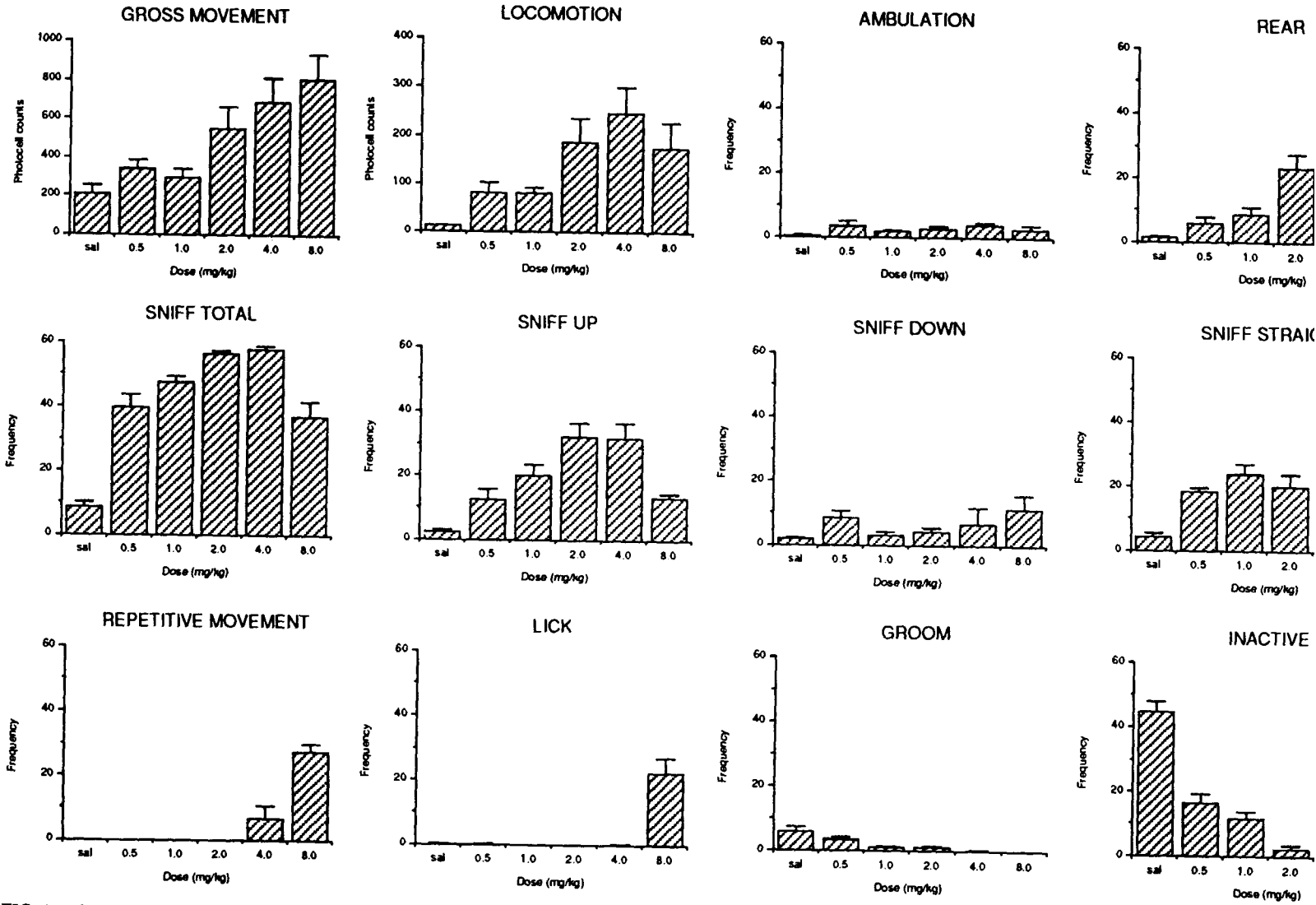


FIG. 1. Effects of various doses of *d*-amphetamine on the behavior of rats in activity boxes. Shown are the number of locomotion (latch) and gross movement (total photoce well as the incidences of 10 behavioral activities scored by the rapid time sampling procedure, over the 1-h period following drug injection. For the automatic measures represent the number of counts. For the behavioral samples, the bar heights represent the mean number of times the behavior was scored (either alone or in combination behavior). SEMs are also shown. Note that the inactive score is the sum of the standing still and lying inactive scores. Significant drug effects ($p < 0.05$) are described in the te dose).

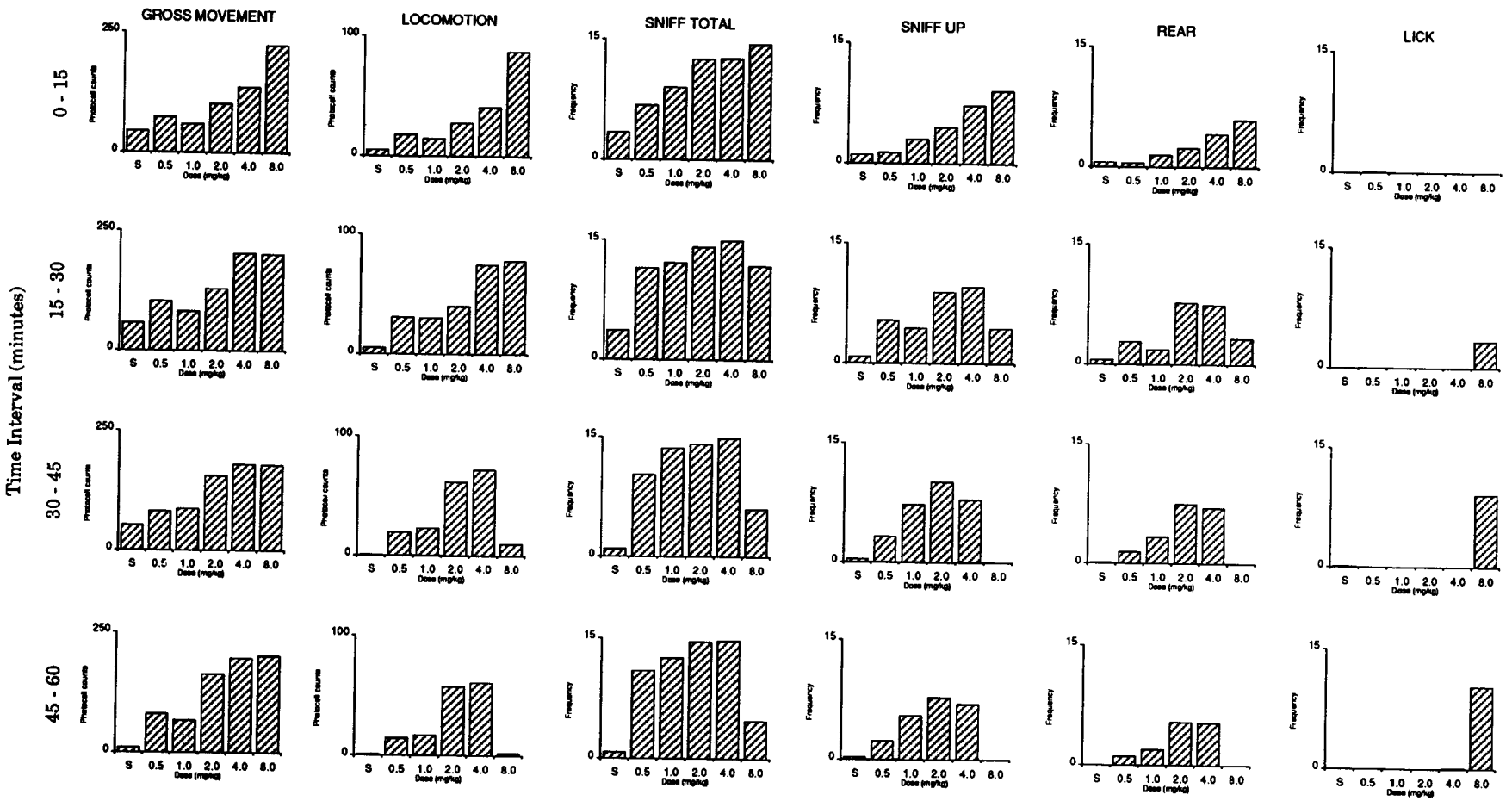


FIG. 2. Time-dependent effects of various doses of *d*-amphetamine on locomotion, gross movement, and selected behaviors. Significant drug effects are described in the text. See Fig. 1. for further details.

Rear. The analysis revealed a significant dose × time interaction, $F(15, 126) = 6.41$, $p < 0.0001$. Rearing was dose dependently increased by *d*-amphetamine (2.0–8.0 mg/kg) during the first 15-min period (Fig. 2). All but the 1.0-mg/kg dose increased the incidence of rearing during the second period. Thereafter, rearing was not significantly enhanced by the lowest dose of drug and was absent after 8.0 mg/kg *d*-amphetamine.

Sniff up. Rearing was often accompanied by sniffing in an upward direction. In fact, the dose-response curves for these two measure were similar (Figs. 1 and 2) and a significant positive correlation was noted at each dose level ($r = 0.76$–0.96, $p < 0.03$–0.0001). A significant dose × time interaction for sniffing up was also noted, $F(15, 126) = 9.68$, $p < 0.0001$. While 2.0–8.0 mg/kg *d*-amphetamine increased the incidence of sniffing up during the first period, all doses were effective during the following 15 min. In the second half of the session, sniffing up was absent after 8.0 mg/kg *d*-amphetamine, while all other doses produced a significant increase.

Sniff down. Sniffing down was the least common form of sniffing. The interaction between dose and time reached significance, $F(15, 126) = 1.77$, $p < 0.05$. The incidence of sniffing down was only altered by *d*-amphetamine in the third period; a significant increase by the highest dose of drug was noted. The sniffing down observed after 4.0–8.0 mg/kg *d*-amphetamine was of a highly repetitive nature during the later part of the session. In fact, repetitive movement and sniffing down were highly correlated in rats administered 4.0 mg/kg *d*-amphetamine (Pearson's $r = 0.91$, $p < 0.002$).

Sniff straight. A significant dose × time interaction was revealed by the analysis, $F(15, 126) = 2.15$, $p < 0.02$. All drug doses increased the incidence of sniffing straight in the first period, while only the 1.0-mg/kg dose was effective in the following 15 min. All but the highest dose of drug increased sniffing straight in the second half of the session (Fig. 1).

Total sniffing. The overall incidence of sniffing was markedly increased by all doses of *d*-amphetamine, although the effects of the 8.0-mg/kg dose were time dependent [dose × time, $F(15, 126) = 7.0$, $p < 0.0007$]. The overall incidence of sniffing was significantly higher in the first half of the session after this dose than during the last two periods (Fig. 2).

Repetitive motion. The 8.0-mg/kg dose of *d*-amphetamine, and to a lesser extent 4.0 mg/kg, produced a highly repetitive form of movements of the forepaws, head, and upper torso (Fig. 1). The two-way ANOVA revealed a significant dose × time interaction for the repetitive motion measure, $F(15, 126) = 24.63$, $p < 0.0001$. Following the highest dose of drug, this behavior first appeared in the second period and reached a peak incidence throughout the second half of the session. A lower incidence of repetitive motion was observed after 4.0 mg/kg during the 30- to 60-min period. The time course of this activity is not shown, but it closely resembled that observed with licking (see Fig. 2).

Lick. Licking was in general of the grid floor and sometimes the cage wall. The two-way ANOVA revealed a significant dose × time interaction, $F(15, 126) = 27.18$, $p < 0.0001$. Licking was produced by the highest dose of *d*-amphetamine; this behavior was first observed in the second period, and the incidence was maximal during the second half of the session (Fig. 2). A strong negative correlation existed for licking and sniffing down after 8.0 mg/kg *d*-amphetamine (Pearson's $r = -0.94$, $p < 0.0005$). Gnawing and biting were not observed.

Groom. The incidence of grooming was reduced or abolished by all but the lowest dose of *d*-amphetamine [dose, $F(5, 42) = 5.39$, $p < 0.0006$; Fig. 1].

Inactive. Significant effects of dose, $F(5, 42) = 63.36$, $p < 0.0001$, and time, $F(3, 126) = 18.32$, $p < 0.0001$, were noted. All doses of *d*-amphetamine reduced the incidence of inactivity (Fig. 1).

SCH23390 and Raclopride vs. Low-Dose d-Amphetamine

In both antagonist studies, 1.5 mg/kg *d*-amphetamine increased latch (locomotion) counts and the incidences of ambulation and rearing (see Table 2 for ANOVA *F* values). Sniffing up was markedly increased, while the frequencies of sniffing down and sniffing straight were not changed. Grooming and inactivity were significantly reduced by *d*-amphetamine (see Fig. 3). Total photocell counts are not presented in either study because the pattern of results was similar to that noted with the latch counts.

SCH23390 dose dependently reversed the behavioral changes produced by this low dose of *d*-amphetamine (Fig. 3). While the lowest dose of drug did not significantly reduce the *d*-amphetamine-induced increase in latch photocell counts, 25–50 μg/kg SCH23390 restored activity to baseline levels.

The rearing and ambulation produced by *d*-amphetamine were in particular sensitive to inhibition by SCH23390 because baseline levels were restored after 12.5 μg/kg. Sniffing up and overall sniffing levels were reduced to control values after 25–50 μg/kg SCH23390. These doses of drug restored the incidence of inactivity to that observed in the control session, while the frequency of grooming increased to basal values after the highest dose of drug. No significant effects on sniffing down and sniffing straight were indicated by the analyses (Table 2).

Raclopride (400–800 μg/kg) also reduced photocell latch counts to control levels, although the drug was less potent than SCH23390 (Table 2 for statistical analyses). These two higher doses of drug also completely blocked *d*-amphetamine-induced increases in ambulation, rearing, sniffing up, and the overall level of sniffing. The statistical analyses for sniffing down and sniffing straight were not significant. The incidence of inactivity was restored to control levels by 400–800 μg/kg raclopride, while the reduction in grooming produced by *d*-amphetamine was not reversed (Fig. 3).

SCH23390 and Raclopride vs. High-Dose d-Amphetamine

The 8.0-mg/kg dose of *d*-amphetamine produced repetitive head and body movements accompanied by sniffing down. The repetitive motion was completely antagonized by 100–200 μg/kg SCH23390, while sniffing down was not completely blocked until administration of the highest dose of drug (Table 2). The overall level of sniffing was also restored to control levels by this drug dose. In contrast, the incidences of inactivity and grooming were lower than in control sessions.

Latch photocell counts, and the incidences of ambulation and rearing, were not influenced by this dose of *d*-amphetamine. However, these behavioral measures were elevated significantly above *d*-amphetamine control levels after 50–100 μg/kg SCH23390. In addition, the number of latch photocell counts and incidence of ambulation were also significantly higher than the 100-μg/kg dose than observed in saline control sessions. A similar stimulation of sniffing straight was observed after various doses of the D_1 antagonist, while the reduction in sniffing up produced by high dose *d*-amphetamine was reversed by all doses of drug.

In summary, these findings indicate that the initial reduc-

TABLE 2

ANOVA TABLES FOR THE EFFECTS OF DA ANTAGONISTS ON *d*-AMPHETAMINE-INDUCED BEHAVIORAL CHANGES

	Low-dose *d*-Amph		High-dose *d*-Amph	
Behavior	SCH23390	Raclopride	SCH23390	Raclopride
df	4, 24	5, 35	5, 35	6, 42
Photocell	7.44	8.39	3.96	8.83
	0.0005	0.0001	0.006	0.0001
Ambulation	4.83	4.40	3.90	2.26
	0.006	0.004	0.007	n.s.
Rear	6.26	5.86	3.16	6.45
	0.002	0.0005	0.02	0.0001
Groom	5.62	8.04	11.85	8.22
	0.003	0.0001	0.0001	0.0001
Inactive	9.08	5.68	19.94	6.56
	0.0001	0.0006	0.0001	0.0001
Total sniff	10.53	6.51	14.17	11.49
	0.0001	0.0007	0.0001	0.0001
Sniff up	10.02	7.62	3.26	5.39
	0.0001	0.001	0.02	0.0003
Sniff down	1.55	0.11	12.82	6.19
	n.s.	n.s.	0.0001	0.0001
Sniff straight	1.18	1.69	4.19	2.13
	n.s.	n.s.	0.005	n.s.
Repetitive motion	–	–	24.21	10.69
	–	–	0.0001	0.0001

Shown are the *F* values (top line) and significance levels (bottom line) for each behavioral category. n.s. is equivalent to $p > 0.05$.

tion in stereotyped behavior induced by SCH23390 (100 μg/kg) is accompanied by a mild behavioral stimulation. A doubling of SCH23390 dose abolishes this effect.

Raclopride (0.2–1.6 mg/kg) antagonized *d*-amphetamine-induced sniffing down (Fig. 4 and Table 2). All doses of raclopride inhibited repetitive motion but a strict dose-dependent relationship was not observed. The 0.2- and 1.6-mg/kg doses completely abolished repetitive motion. The analyses for locomotion, ambulation, rearing, and sniffing straight did not reveal significant drug effects (Table 2). Sniffing up was increased above *d*-amphetamine, but not saline, levels after certain doses of raclopride (0.1, 0.2, and 0.8 mg/kg). The highest dose of the D_2 antagonist restored the overall sniffing score and the incidence of inactivity to control levels. Grooming was not reinstated.

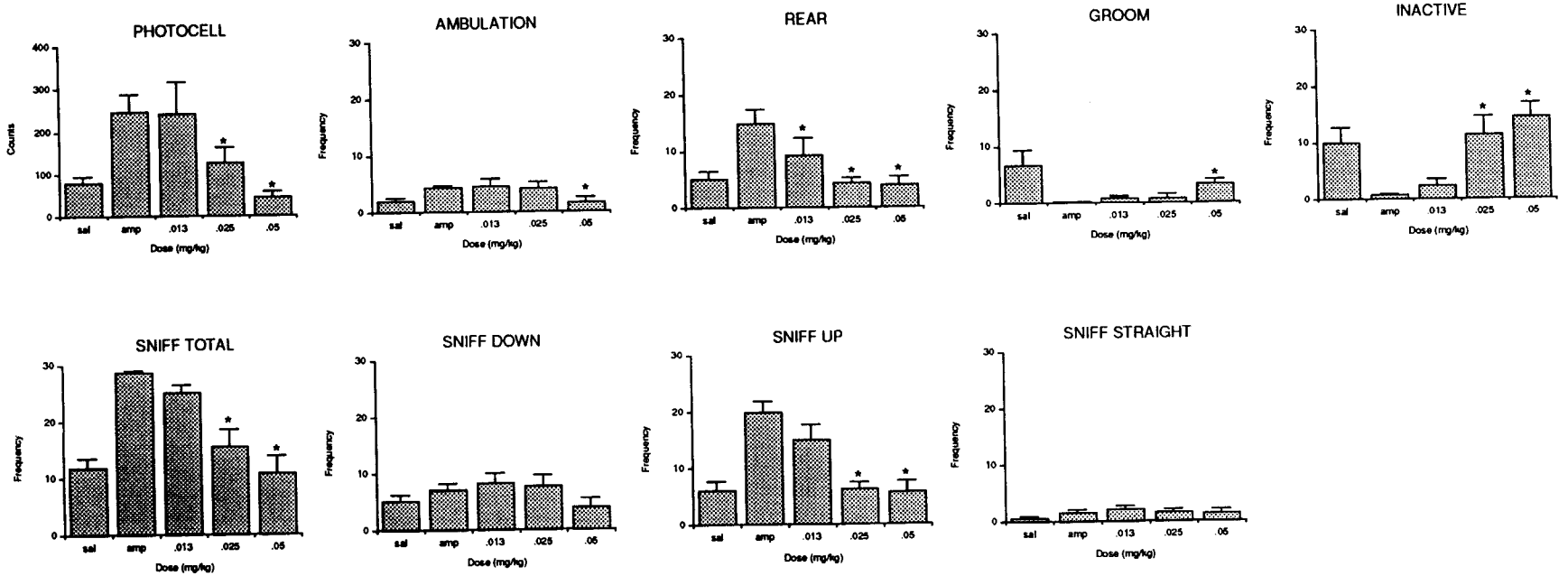
DISCUSSION

On the basis of an extensive review of the literature, Lyon and Robbins (22) formulated the following general hypothesis for the behavioral effects of *d*-amphetamine: "The action of amphetamine on behavior is such that as the dose response within the central nervous system increases, the repetition rate of all motor activities will increase with the result that an animal will tend to exhibit increasing response rates within a decreasing number of response categories." The detailed profile of *d*-amphetamine-induced behavioral changes we demonstrated in the present study are certainly consistent with this hypothesis.

Low doses of *d*-amphetamine (0.5–4.0 mg/kg) increased the automated measures of activity, as well as the incidences of rearing, sniffing, and ambulation. Although all forms of sniffing were significantly increased by *d*-amphetamine, only sniffing up showed a clear dose dependency. The occurrence of this form of sniffing was highly correlated with rearing. The incidences of inactivity and grooming were markedly reduced by low doses of *d*-amphetamine. As pointed out by Lyon and Robbins (22), grooming should be easily disrupted by *d*-amphetamine because it represents an organized pattern of responses and tends to occur in bouts separated by relatively long interbout intervals.

A different pattern of behavioral changes were observed after 8.0 mg/kg *d*-amphetamine. Locomotion (latch) counts were markedly elevated early in the session and then declined rapidly. The incidences of rearing and sniffing up reached maximal levels at the same time as the locomotion measure, but these behaviors were not observed in the last half of the session. These changes corresponded with the appearance of licking, intense sniffing down, and highly repetitive head and limb movements. A similar switch in behavioral pattern at later time intervals following high doses of *d*-amphetamine has been noted by other investigators (18,38). Presumably, as the concentration of *d*-amphetamine in the brain increases the range of motor acts becomes more restricted due to a form of behavioral competition that only allows those responses with short, discrete components to be elicited (22). Thus, the rearing, sniffing up, and locomotion seen earlier in the session (and with lower drug doses) is replaced by the repetitive sniffing down (which contains small and rapid upward movements of the snout) in the absence of ambulation (small limb movements are noted). The late onset of these behaviors appears to correspond well with the maximal release of striatal DA induced by *d*-amphetamine, as measured by microdialysis techniques (17,39).

SCH 23390


PHOTOCELL
Counts
AMBULATION
Frequency
REAR
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GROOM
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INACTIVE
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Dose (mg/kg)

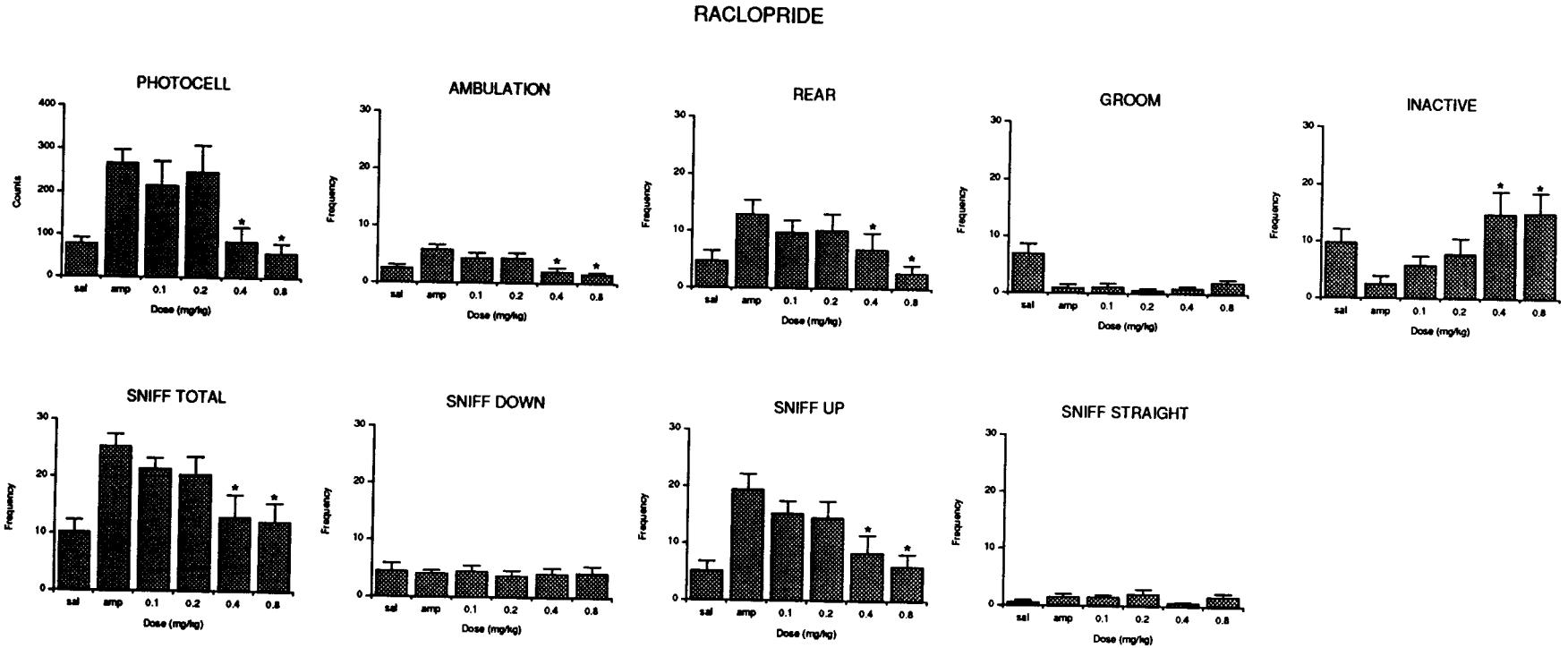
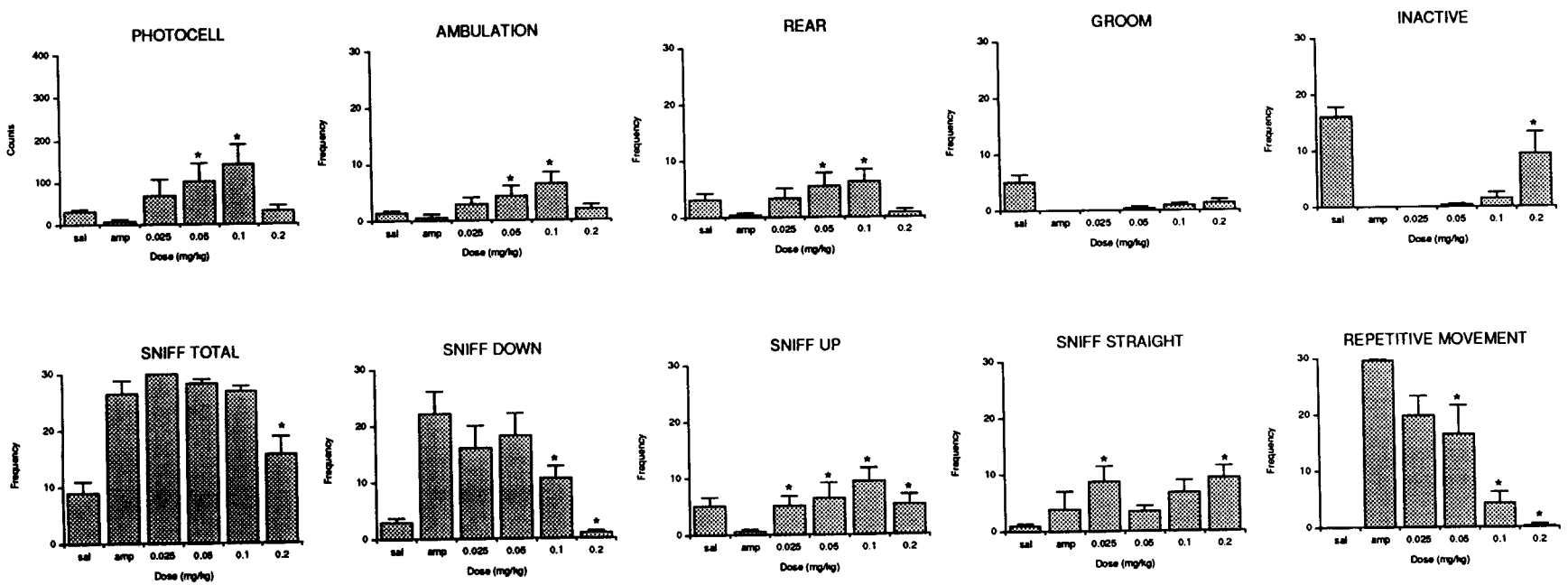


FIG. 3. Effects of SCH23390 and raclopride in combination with 1.5 mg/kg *d*-amphetamine on the behavior of rats in activity boxes. Bar heights reflect the mean latch and photocell counts (±SEM), and the mean incidences of eight behavioral activities (±SEM), measured over a period of 30 min. Significance levels for test doses: $*p < 0.05$ vs. *d*-amphetamine control ($n = 8$ per study).

SCH 23390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Counts
AMBULATION
REAR
GROOM
INACTIVE
SNIFF TOTAL
SNIFF DOWN
SNIFF UP
SNIFF STRAIGHT
REPETITIVE MOVEMENT
Frequency
sal
amp
0.025
0.05
0.1
0.2
Dose (mg/kg)

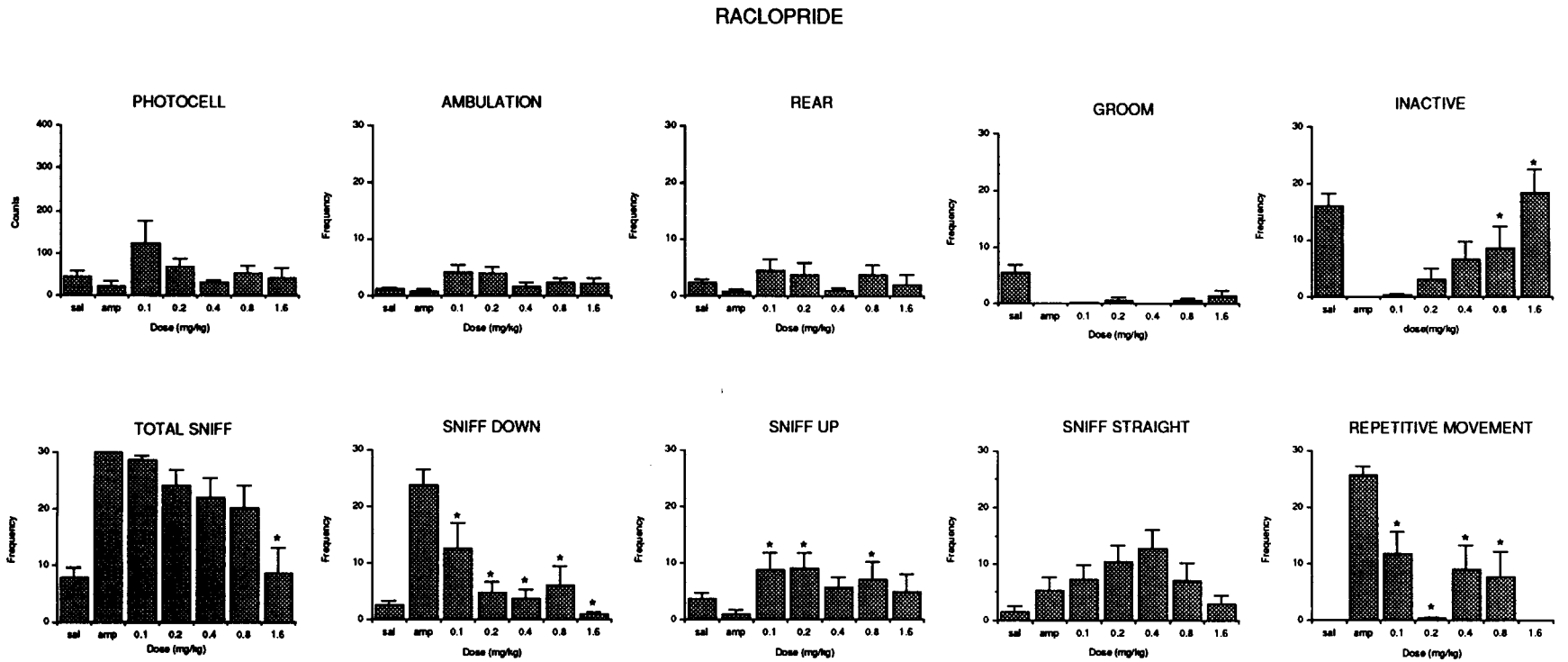

FIG. 4. Effects of SCH233900 and raclopride in combination with 8.0 mg/kg *d*-amphetamine on the behavior of rats in activity boxes. Bar heights reflect the mean scores (±SEM) for photocell counts and nine behavioral activites measured over a period of 30 min. Significance levels for test doses: $*p < 0.05$ vs. *d*-amphetamine control ($n = 8$ per study).

This initial study provided information for the selection of dose and pretreatment time for work with the selective DA antagonists. In the latter studies, 1.5 mg/kg *d*-amphetamine produced clear increases in locomotion counts, ambulation, rearing, and sniffing up. However, sniffing straight was not influenced in these studies [see also (3)]. Rather marked differences were noted between the effects of 8.0 mg/kg *d*-amphetamine in the antagonist study and in the initial dose-response experiment. Little licking was observed in the former; the predominant behavior in these, and our other studies [(3); see also (13)], was an intense sniffing down accompanied by highly repetitive head and limb movements. Sniffing down was only noted to some extent in certain individuals participating in the dose-response study.

It cannot be argued that the failure to observe licking in the antagonist studies is due to the repeated-measures design and a tolerance developing to this behavior [cf. (30)]. We have always observed little licking in this type of experiment when animals receive their first drug injection. Moreover, we have observed a high level of licking in some rats participating in a repeated-measures design experiment (40). In this study, animals, like those in the present dose-response experiment, were habituated immediately prior to drug injection. In all our other work, animals only received habituation sessions on the days prior to drug administration and, understandably, exhibited higher basal levels of activity. The higher intensity behavior (i.e., licking) observed in rats habituated just prior to drug injection may reflect rate-dependent effects of *d*-amphetamine; that is, the drug increased a low rate of responding to a greater extent than a high rate (10,22). Whatever the relevant factor, the present findings indicate that the form of habituation may influence subsequent effects of *d*-amphetamine.

Both SCH23390 and raclopride dose dependently reduced the behavioral activation produced by 1.5 mg/kg *d*-amphetamine with the D_1 antagonist being approximately 10-fold more potent. The behavioral changes produced by this dose of *d*-amphetamine (increased locomotion, rearing, and sniffing up) were antagonized at almost identical doses of SCH23390. A similar consistency was noted with raclopride and, in other work, following administration of haloperidol (3). This suggests that the *d*-amphetamine-induced behavioral changes are subserved by a common neural mechanism. The locomotor hyperactivity, rearing, and sniffing produced by low doses of *d*-amphetamine are thought to be mediated by enhanced dopaminergic function in the nucleus accumbens (2,6,15,27,39).

Both DA receptor antagonists increased the incidence of inactivity to levels observed in control sessions. In fact, our sampling procedure indicated that animals administered 0.05 mg/kg SCH23390 exhibited a pattern of behavior indistinguishable from that observed in control sessions. However, this was not the case with raclopride or, for that matter, haloperidol (3). In contrast to SCH23390, these drugs failed to restore grooming to control levels. This finding would suggest that raclopride and haloperidol do not act specifically to block the behavioral activation produced by *d*-amphetamine but may exert other actions (e.g., increasing muscle tone) that prevent the appearance of a complicated behavioral act such as grooming. Higher doses of SCH23390 than used in the present study probably also increase muscle tone (11).

SCH23390 and raclopride equipotently blocking the repetitive head/body movements and intense sniffing down produced by 8.0 mg/kg *d*-amphetamine. We can offer no explanation for the unusual dose-response relationship observed with raclopride on repetitive motion. SCH23390 was slightly less potent in antagonizing these behaviors than in blocking the behavioral activation produced by the low dose of psychomotor stimulant. In contrast, raclopride was more potent in antagonizing the repetitive sniffing down than in inhibiting the low-dose locomotor activation. The latter finding contrasts with the report that higher doses of raclopride are required to inhibit apomorphine-induced oral stereotypies than the locomotor activation produced by the direct-acting DA agonist [(26); but see (4)]. The abilities of SCH23390 and raclopride to abolish the stereotyped behavior produced by *d*-amphetamine is almost certainly due to blockade of DA receptors in the striatum (6,15).

When administered with 8.0 mg/kg *d*-amphetamine, high doses of SCH23390 and raclopride reduced or blocked the overall level of sniffing and produced inactivity. However, grooming was not reinstated. A low dose of SCH23390 (0.1 mg/kg) both blocked the repetitive behavior produced by 8.0 mg/kg *d*-amphetamine and increased locomotion (latch counts and ambulation) and sniffing straight to levels above those observed in the control session. This behavioral stimulation was different from that observed with low doses of *d*-amphetamine because rearing and sniffing up were not enhanced. We obtained similar findings with haloperidol (3), but such effects were not observed with raclopride. Enhanced locomotion following combined administration of neuroleptic drugs and high-dose *d*-amphetamine has previously been reported (24,28).

A number of points concerning our experiment approach merit attention. First, there were discrepancies between our automated measures of locomotion (latch counts) and gross movement (total photocell counts). In the dose-response study, the latch count revealed clear time-dependent effects of the highest dose of *d*-amphetamine that were confirmed by the behavioral observation procedure. Although animals were initially active, they displayed highly repetitive licking and sniffing in a restricted location in the last half of the session. However, the total photocell counts did not reflect these changes. Animals could still register high total photocell counts when engaged in stereotyped behavior because they would continually break one photocell beam. The slight negative correlation for latch and total photocell counts observed in the saline condition also illustrates how stationary activities such as grooming influence the gross movement but not locomotion measure. These findings indicate that simply totaling the number of beam breaks in an activity box with multiple photocells is not an accurate estimation of locomotion. A number of other investigators have pointed out that observed changes in behavior do not correlate well with automatic measures (13,20). Moreover, Sahgal and colleagues (34) also demonstrated potential interpretational difficulties from just using a total photocell count. Their findings, along with those of the present study, argue for a procedure where total beam breaks and latch counts are measured simultaneously. This is easy to arrange using computer software. In addition, we reduced the influence of small, rapid movements by setting our photocell detectors to ignore beam breaks that occur within 0.5 s of the previous interruption.

Locomotor activity (ambulation) was also assessed by the observation procedure. In all studies, the incidence of ambulation was low and only correlated with the automated measure of locomotion in certain instances. Because rats tended to exhibit short-lasting bursts of rapid ambulation when administered *d*-amphetamine, this result is not surprising. These bursts of ambulation were not readily picked up by sampling at a specific time and, therefore, it might be better to adopt a

procedure whereby the behavior is classed as present if it occurs during the 5-s period [cf. (13)]. However, such a procedure would need to be adopted for scoring all behavioral categories and, because we have found rapid alterations in behavior to be a typical result of low-dose *d*-amphetamine administration, the skills of a touch typist would be required to accurately record the various behaviors and combinations of behavior occurring during even such a short time interval. As these skills are not often found in a research laboratory, observation at frequently repeated time points ensures interobserver reliability. It should also be pointed out that our findings might best reflect the time animals spend engaging in each activity. Although animals may initiate many bursts of ambulation, the total time this activity takes up appears to be lower than the total duration of rearing when lower doses of *d*-amphetamine are administered. On the other hand, we observed high ambulation scores using this technique when monitoring the activity of apomorphine-treated rats. These animals spend periods of time either running around or moving methodically around the activity box (40).

Overall, the time sampling procedure provided highly detailed records of the nature and time course of the behavioral response to *d*-amphetamine. The necessity for such "activity prints" of psychoactive drugs has previously been emphasized (35). Clear differences between the activating effects of low-dose (rearing, sniffing up) and high-dose (licking, sniffing down, repetitive movements) *d*-amphetamine were demonstrated. In addition, we were able to show that phases of both forms of activation occur when a high dose of the stimulant is administered. The observation procedure has also allowed us to demonstrate subtle differences in the inhibitory effects of SCH23390 and raclopride. SCH23390, in general, seems to exert a more "normalizing" effect on both low- and high-dose *d*-amphetamine-treated rats than does raclopride. Such subtle effects could not be detected using standard activity monitoring and rating scale procedures. The approach we adopted in detailing behavioral profiles is similar to that used by other investigators who have relied upon the Digiscan Activity Monitoring system for automatic assessment of a variety of activity measures (35,36). This apparatus has also been useful in illustrating subtle differences in drug effect that are not revealed by standard activity monitoring systems (35). Clearly, there are advantages to both approaches; the latter provides a more detailed assessment of certain aspects of movement, while the observation procedure provides a better indication of what behavioral activities are actually occurring.

In conclusion, we demonstrated the use of a computer-supported behavioral observation technique readily combined with two forms of automatic activity monitoring. This procedure permits the study of drug effects on multiple-response topographies and enables the precise evaluation of qualitative as well as quantitative changes in behavior. The greater investment in time required for such a procedure should be well compensated for by the more precise information the technique provides.

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