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The Effect of Apomorphine on the Open-Field Behavior of Rats: Alone and in Pairs[1]

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CHOW, H. L. AND C. H. M. BECK. *The effect of apomorphine on the open-field behavior of rats: Alone and in pairs.* PHARMACOL BIOCHEM BEHAV 21(1) 85–88, 1984.—Male rats were observed in the open-field while alone and while in pairs in an alternating series of trials. The trials extended over a 78 min session following injections of either saline (0.9%) or apomorphine (5.0 mg/kg, IP) into the observed member of each pair. Contrary to the literature on apomorphine stereotypy, apomorphine did not induce continuous sniffing of the environment and continuous gnawing in most rats. Sniffing of the environment remained at normal levels but there was an increase in nodding the head in the vertical plane while keeping the snout close to the floor. Apomorphine-induced hyperactivity was attributed to two factors: a sustained increase in the duration of bouts of locomotion and a failure of the frequency of bouts of locomotion to habituate to novelty. Apomorphine eliminated all social behavior directed toward the other rat, however apomorphine rats showed they were sensible to the presence of the other by increasing their locomotion and rearing when the partner was introduced.

Apomorphine Rats Open-field Stereotypy Social behavior

WHILE it is well established that high doses of apomorphine (0.5 to 5.0 mg/kg) produce hyperactivity and stereotyped behavior in the lone rat in the open-field, there is some disagreement as to the precise nature of the behaviors. Observation of rats for 10s periods every 10 minutes revealed that apomorphine produced significant increases in locomotion, sniffing, licking, gnawing, and head-down posture [3]. Utilizing a combination of continuous recording and recording in scans at 5 min intervals, large individual differences were noted between rats of the same strain in the degree to which apomorphine generated climbing, sniffing or gnawing [15]. However, all rats, including members of two substrains, consistently exhibited head-down, or what the authors called snout contact [15]. Head-down, rather than sniffing or gnawing, was judged to be the most appropriate measure of apomorphine stereotypy because of its generalizability across individuals and its persistence over the time course of the drug effect. The issue is significant since gnawing and sniffing are key constituents in the commonly used stereotypy scales for dopamine agonists [1]. Disagreement over the time course of stereotypic behaviors is also evident. The time courses of apomorphine-induced locomotion and gnawing have been found to peak sequentially [6] or to be concurrent [3]. The typical scale of intensity of stereotypy assumes an initial period of hypermobility superseded by a period of declining locomotion and increased gnawing and sniffing [1].

Following the methodology which treated frequency and duration measures separately [11], the present study documented the time course of apomorphine-induced behavior in some detail by recording the frequency and duration of 16 behaviors. In particular, we were interested in distinguishing between whisker twitching (sniffing), holding the head close to the floor (head-down), repetitive up and down head movements (nodding), making grating noises on the floor (gnawing) and on the walls (rear-gnawing) with the teeth. To assess the persistence of the stereotypy, the injected rats were challenged by the periodic placement of noninjected rat into the open-field. Intense stereotypy should prevent the apomorphine rats from responding socially, resulting in the social withdrawal typical of the primate under the influence of dopamine agonists [13,14].

METHOD

Male Sprague Dawley rats, raised at the University of Alberta Farm, weighing 260–310 g were housed individually in a colony room maintained at 19±1°C and 51% humidity on a reversed lighting cycle with lights off from 0900 to 2100 hr. The animals were adapted to handling during the two weeks

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prior to testing. Before testing began, the animals were randomly paired and the pairs assigned to two groups of ten pairs per group. One animal of each pair was designated as the experimental animal and the other as the unobserved animal.

In addition to the test injection given 5 minutes prior to behavioral testing, all experimental rats received a conditioning injection of saline (2 ml/kg, 0.9% NaCl, IP) 3 days before the test day in order to reduce intersubject variance in reaction to the novelty of being injected [12]. The test injection for the saline group was again saline and for the apomorphine group was apomorphine hydrochloride (5 mg/kg, SC) dissolved in saline immediately before use. The unobserved rats were not injected.

Behavioral observation was begun after 5 minutes of adaptation of the experimental rat to the test box immediately following the second injection. The experimental rat was observed through a one-way mirror as it moved about the test box, a black wooden open-field 55 cm by 66 cm by 64 cm high with 30 squares drawn on the floor. The test room was illuminated by a 45 watt red light bulb hung 120 cm above the center of the floor of the open field. The floor and walls were scrubbed clean after the testing of each rat.

The behavior of the experimental rat in the open-field was recorded during 18 two-minute observation periods spaced over a 78 minute session. The observation periods were clustered into six trials with three periods in each trial. The intertrial interval was five minutes and the interperiod interval within trials was one minute. The experimental rat's unobserved partner was present in the open-field during the second, fourth, and sixth trial referred to as *pair* trials or collectively as the *pair* condition. During the intertrial intervals and during the remaining three trials, the first, third and fifth trials, referred to as *alone* trials or as the *alone* condition, the experimental rat was alone in the field.

The observer recorded the experimental rat's behavior continuously throughout each observation period using a microprocessor. The behaviors logged were: *line-cross*, the rat's forequarters moved across a square; *locomote*, a bout of consecutive line-cross responses; *rear*, raising the forepaws so that they were not touching the floor or the unobserved animal; *sniff-field*, sniffing the environment as indicated by nose and whisker twitching while otherwise sitting still; *self-groom*, licking, combing, mouthing or scratching itself; *allogroom*, grooming the unobserved rat in a nonaggressive manner; *allosniff*, sniffing the unobserved animal; *aggress* and *submit*, included all such behaviors as described by Miczek [10]; *head-down*, holding the head below the level of its body, without sniffing or scanning movements while stationary; *nod*, moving the head rhythmically forward and backward in a vertical plane while otherwise still; *gnaw*, grinding the teeth audibly on the floor of the box while nodding; *rear-gnaw*, gnawing the wall of the box while rearing; *leap*, leaping vertically off the floor; *jumping*, a bout of consecutive leaps; *inactive*, remaining immobile while not exhibiting any of the other behaviors. The frequency of bouts of each behavior of each animal in each trial and the mean duration of the bouts of each behavior of each animal in each trial were tabulated from the logged data. All testing was done by a trained observer who was ignorant of the injection history of the experimental animals. Interjudge correlations with an independent observer's scores were greater than 0.90, $p<0.001$.

The Michigan Terminal System Revised SPSS programs were used in all computations. For each behavior, frequency scores and transformed mean duration scores (X=square root of X+1) were subjected to ANOVA of group and trial effects and interactions both within and across conditions ($p<0.05$). Duncan multiple range tests ($p<0.05$) and two-tailed t-tests ($p<0.05$) were used to dissect the ANOVA effects.

RESULTS

Mean frequency and mean duration scores of the saline and apomorphine groups over the six trials are presented for selected behaviors (Figs. 1 and 2). Data on rear-gnaw, jump and leap are not shown since these behaviors produced no significant effects. Line-cross data are omitted since the pattern of significant effects was identical to that obtained with frequency of locomote. Where the pattern of significant effects are similar for frequency and duration measures, only frequency measures are presented.

Whereas apomorphine rats increased their duration of locomotion relative to saline rats in both the alone and the pair condition, this was true of frequency of locomotion only in the alone condition (Fig. 1). The apomorphine rats did not locomote more frequently than the saline rats in the pair trials because although the introduction of a partner increased the frequency of locomotion in the apomorphine rats as well as in the saline rats, the increase was greater in the saline rats. A significant group × trial interaction in the alone condition for the frequency of locomotion was the result of a greater decline in locomotion across alone trials in the saline rats than in the apomorphine rats.

Saline rats sniffed the environment more than apomorphine rats as indicated by their longer duration of sniff-field in the alone condition and higher frequency of sniff-field in the pair condition (Fig. 1). Two effects accounted for these differences. The first was the saline rats' decrease in frequency of sniff-field and increase in duration of sniff-field across trials in the alone condition. The second was the partner-generated increase in the frequency of sniff-field and decrease in the duration of sniff-field in the saline rats. The apomorphine rats' sniffing of the environment was not significantly altered by either the condition effects or the trial effects within conditions.

The apomorphine rats reared less frequently than did the saline rats in both the alone and pair conditions (Fig. 1). Although this effect in the pair condition was attributable to more frequent rearing by the saline rats throughout the pair trials, their enhanced rearing frequency in the alone condition arose solely from the initial alone trial (Fig. 1). Introduction of a conspecific enhanced the frequency of rearing in both the saline group and the apomorphine group. A significant group by trial interaction of rearing frequency in the alone condition was reflected in a significant trial effect across alone trials in the saline group compared to a nonsignificant value in the trial effect for the apomorphine group. The two groups did not differ in the duration of rearing.

The apomorphine rats showed very little inactivity throughout. The saline rats exhibited more frequent inactivity than the apomorphine rats only during the alone condition (Fig. 1). The effect was due to the increase in inactivity of the saline rats over trials. Similar results were obtained for the duration of inactivity.

The apomorphine rats self-groomed less frequently than the saline rats in the alone condition and pair condition (Fig. 1). The same was true for duration of self-grooming.

Although the apomorphine rats gnawed more frequently

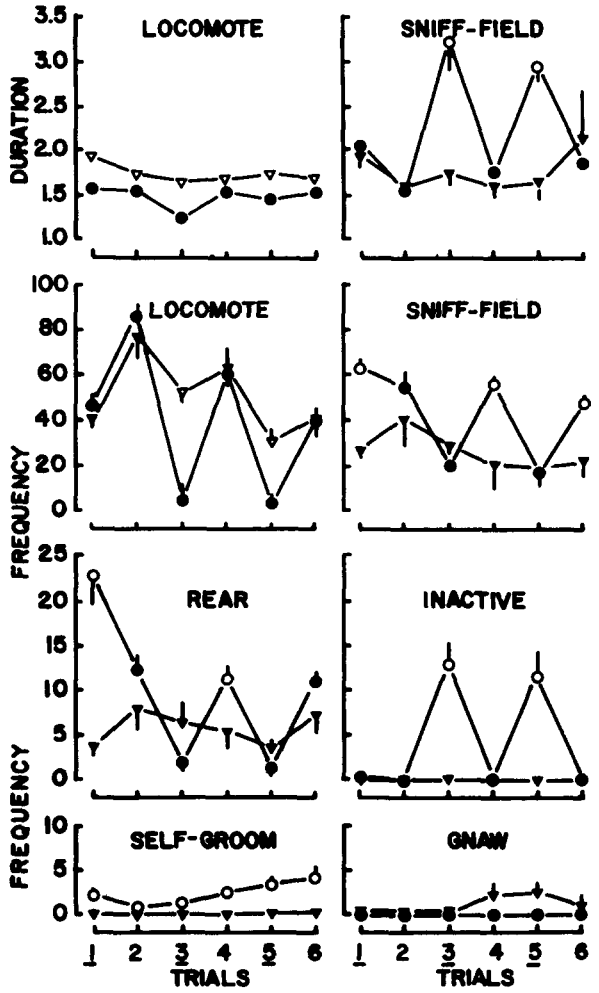

FIG. 1. Mean and S.E.M. of frequency of 6 behaviors and duration in s (x=square root of x+1) of 2 behaviors over 6 successive trials for saline rats (circles) and apomorphine rats (triangles). Filled circles or triangles indicate a nonsignificant group difference on a particular trial. Open circles or triangles indicate a significant difference (Duncan test, $p<0.05$). Trials 1, 3, and 5 (underlined) are alone trials, trials 2, 4, and 6 are pair trials.

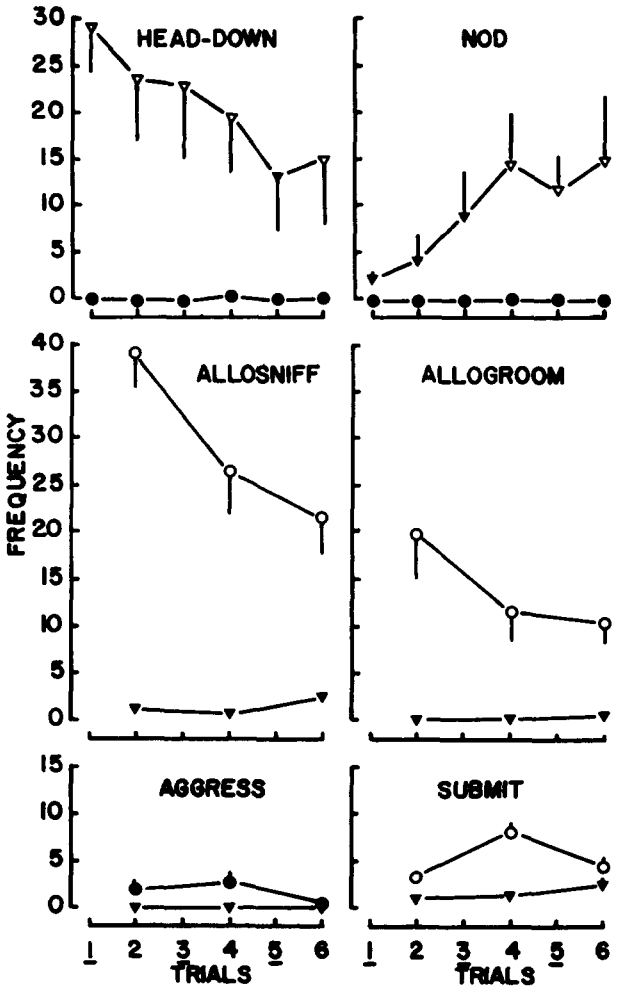

FIG. 2. Mean and S.E.M. of frequency of 6 behaviors over 6 successive trials for saline rats (circles) and apomorphine rats (triangles). Particulars as in Fig. 1.

and longer than the saline rats on the later trials, the group effects were not significant (Fig. 1). A barely significant group by trial interaction in the frequency of gnawing in the alone condition was a reflection of this. The results for duration of gnawing were similar.

Apomorphine induced two behaviors not seen in saline-injected rats, namely head-down and nod. Apomorphine rats showed more frequent head-down in both the alone condition and in the pair condition (Fig. 2). The introduction of a partner had no effect on head-down measures. An identical pattern of results was obtained for the duration of head-down.

Apomorphine rats nodded more frequently than saline rats in the alone and in the pair conditions (Fig. 2). The same was true of the duration of nod. Although the apomorphine rats' nodding increased over successive trials, the across trial changes were significant only for the duration of nod in the alone condition. The only significant group by trial interaction was for nod duration in the alone condition. Direct comparison of alone and pair condition scores on nodding did not yield any significant effects of adding a partner.

Apomorphine greatly reduced direct measures of social behavior (Fig. 2). The apomorphine compared to the saline rats exhibited less frequent allosniffing, allogrooming, aggression, and submission. Significant group by trial interactions for frequency of allosniffing, allogrooming and of submitting reflected significant trial effects for the saline group's frequency of allosniffing, allogrooming and submitting and the lack of the same for the apomorphine rats. The group main effects for the duration of these behaviors were similar to those for frequency. Trial effects and interactions for the duration of these behaviors were not significant.

DISCUSSION

Previous studies of the open-field behavior of rats have tested alone and social conditions separately in an uninter-

rupted fashion [5, 8, 11]. The results of the present study replicated those findings in normal rats for frequency and duration of locomotion, sniffing the environment and self-grooming. Apomorphine induces increases in automated activity counts in the lone rat [3, 4, 6]. Our data suggest that such hyperactivity may be dissociated into two factors. The first is an increase in the frequency of bouts of locomotion in agreement with [6]. This effect is apparent only if the control animal is alone in a familiar environment. The second is an increase in the duration of bouts of locomotion irrespective of environmental novelty or social conditions. This factor has not been reported previously.

Although others [3,16] have noted that apomorphine eliminates self-grooming, this study is the first to show that the deficit is measurable in both duration and frequency when direct comparisons are made with normal rats.

In contrast to the present results, apomorphine has been reported to induce significant increases in gnawing and sniffing [1, 2, 3, 6, 7]. These data were obtained using the same dosage [3, 6, 7], the same injection route [3, 6, 7] and the same rat strain [6,7] as the present study. We used the grating noise made by the raking teeth as a sign of gnawing as did Ljungberg and Ungerstedt [6,7]. The apomorphine rats gnawing consists not of biting, but of a scraping of the lower incisors, which in our study produced scouring and splintering of the wooden floors and walls. The rats of Ljungberg and Ungerstedt [6,7] gnawed only the edges of holes in a plastic floor and so had less gnawable substrate than did the rats in the present study. The differences in gnawing may be accounted for by substrain differences in the proportion of rats induced to gnaw by apomorphine [16]. The failure to observe an increase in sniffing in the present study may have been due to our differentiation of nodding from the whisker twitching and variable head movements characteristic of sniffing. Nodding has been previously been observed but not quantified in apomorphine rats [1,16].

The apomorphine rats in the present study severely reduced not only agonistic displays but also affiliative activity such as sniffing or grooming the partner. Thus the rats exhibited a social withdrawal similar to that induced by stimulants in man and monkeys [13,14]. Although the drugged rats ignored the partner, they were sensible to the other's presence as indicated by the increased rearing and locomotion of the apomorphine rats following the introduction of a partner. This effect has also been observed in primates [13,14]. Apomorphine-induced fighting was not observed by us, although it has been reported by others using the same strain of rats [15], the same dose of apomorphine [9,15] and the same sized arena [15]. Preliminary observations from this laboratory, to be fully documented in a subsequent report, indicate that when both animals are injected as was the case in earlier rat studies [9,15], that fighting ensues. The present study employed a noninjected conspecific to conform to the paradigm used in the monkey and human literature [13,14].

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