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Effects of Rating Parameters on Assessment of Neuroleptic-Induced Vacuous Chewing Movements

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EGAN, M. F., J. FERGUSON AND T. M. HYDE. *Effects of rating parameters on assessment of neuroleptic-induced vacuous chewing movements.* PHARMACOL BIOCHEM BEHAV **53**(2) 401–410, 1996.—Long-term administration of neuroleptics to rats produces a syndrome of vacuous chewing movements (VCMs). The validity of the VCM syndrome as a model for tardive dyskinesia (TD) in humans is unclear. This is due, in part, to inconsistencies between studies. Methods for rating VCMs have varied markedly and could account for the inconsistencies. The purpose of this study was to evaluate the importance of the different methods on VCM scores. The effects of habituation and length of rating sessions were examined in rats habituated for 2 min, 1 h, or several hours over 4 days, compared to unhabituated rats. Ratings with and without habituation were highly correlated, as were ratings from 2- and 5-min observation periods. Ratings from neuroleptic-treated rats in restraining tubes, however, were significantly correlated with unrestrained ratings only following several hours of habituation. Locomotor activity was not correlated with VCM scores. These results suggest that habituation to open cages is not an important factor in assessing VCMs. Use of restraining tubes, however, may alter scores. The lack of an habituation effect or of a relationship between activity and VCMs suggests that locomotor and oral behaviors are not necessarily in competition. Restraining rats to rate VCMs does not appear to be necessary and could alter the neurobiology of VCMs.

Vacuous chewing movements Habituation Haloperidol

TARDIVE dyskinesia (TD) is a common and sometimes debilitating movement disorder affecting 25 to 30% percent of patients treated chronically with neuroleptic medications (10). The neurobiological basis of TD has been explored using several different animal models. One commonly used model is the syndrome of vacuous chewing movements (VCMs) that develops gradually in rodents during long-term neuroleptic treatment (26). This model is controversial for several reasons (30). First, some investigators have failed to observe increased VCMs during chronic haloperidol treatment (3,11,12,19,20), or have seen them only after intermittent treatment (6). Second, studies of the response of VCMs to several pharmacological challenges have yielded inconsistent results. An acute increase in haloperidol dose, for example, may (7a) or may not (27,28) suppress VCMs. Effects of cholinergic manipulations are also variable (24). Third, VCMs have been noted after a single intraperitoneal injection of haloperidol (23). Such inconsistencies raise significant questions about the validity of this model. Discrepancies could be due, in part, to different parameters used when counting VCMs [see (30) for review]. Given the enormous potential for using the VCM syndrome to study the neurobiology (4,13,18b,21) and treatment (9,2,12) of TD, resolution of these issues is important. Understanding parameters that affect VCM ratings could improve consistency between studies.

A number of methodological variables differ among published reports and could affect the assessment of VCMs. Habituation periods, in particular, have varied widely. Typically, before each rating session, animals are acclimatized to rating cages for 2 min (7a,b), 10 min (13), 30 min (12), or 40 to 60 min (15,24,26–28). Repeated sessions over several days have also been described (5,23). In a related issue, the length of time over which VCMs are evaluated has varied from 10-s epochs over 5 min (5) to 2-min (7a), 5-min (13,28), 6-min (3), 10-min (16), and 15-min periods (24).

Habituation periods may be useful due to a purported in-

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verse relationship between locomotor activity and oral movements (12,14,19). Jaw movements quantified using small cylindrical restraining tubes are not elevated during chronic neuroleptic treatment (3,11,12,19,20), even though VCMs rated in observation cages are elevated (12). Lesions that reduce oral activity increase apomorphine-induced locomotor activity (14). It has been argued that VCMs and/or oral activity are competing behaviors with exploratory or general motor activity. Neuroleptics, due to their sedating or cataleptic effects, could induce a nonspecific increase in oral activity secondary to reduced general motor activity. Alternatively, chewing movements in control animals (12) could be elevated due to the effects of stress, despite efforts at habituation to restraining tubes.

The current study examines the effects of several habituation procedures on VCM ratings. The purpose is to describe parameters that produce consistent VCM ratings over time that can be applied to pharmacological studies of this syndrome. A second, related goal is to assess the relationship between VCMs and locomotor activity in haloperidol-treated animals. We hypothesized that VCM scores were inversely related to general locomotor activity. This was tested indirectly by examining the correlation between activity and VCM scores. A significant inverse correlation between motor behavior and VCM scores would be evidence for the notion that these behaviors are in competition.

METHOD

Animals

Two separate groups of animals were used to examine the effects of habituation on VCMs. The first group included 65 Sprague–Dawley male rats initially weighing 140–160 g housed at a constant temperature of 25°C, in groups of two, under a 12 L : 12 D cycle (lights on at 0700 h; lights off at 1900 h) with free access to food and water. Animals were initially divided into two groups with the control group (n = 12) receiving vehicle injections (donated by McNeil Pharmaceuticals, Spring House, PA) and the haloperidol group (n = 53) receiving intramuscular treatments of haloperidol decanoate (McNeil Pharmaceuticals) at 28.5 mg/kg (the equivalent of 1.0 mg/kg/day unconjugated haloperidol) every 3 weeks for 8 months and then withdrawn for 5 months.

A second group included 66 female Sprague–Dawley rats initially weighing 140–160 g. They were housed and treated similarly to the males with a control group (n = 10) and a haloperidol-treated group (n = 56). Injections were administered every 3 weeks for 7 months, followed by a 4-month withdrawal period. Both male and female groups were rated for VCMs once every 3 weeks for 2 min as described below. No ratings were performed for at least 4 weeks prior to the current study.

Behavioral Assessments

Vacuous chewing movements were counted during rating sessions lasting 2 or 5 min. Only jaw movements occurring independently of eating, gnawing, or grooming were counted and recorded. VCMs were defined as individual vertical jaw movements not directed at any stimuli and infrequently accompanied by tongue protrusions. A second type of movement, bursts of several chewing movements associated with a tremor of the jaw with or without bruxism and/or head tremor that lasted up to 10 s were also observed and counted seperately. Distinct chewing movements during bursts were included in the VCM total. Number of episodes of bursts were not included in analyses due to low frequency. All rating sessions were conducted in a 40 × 30 × 20 cm clear plastic cage placed on a rotating platform permitting visualization of the subjects' jaw at all times. In previous studies, we have rated other groups on a stationary surface with a mirror behind the observation cage, permitting visualization of the mouth at all times. Comparison of ratings from these earlier studies with the current results suggests that platform rotation has little effect on VCM ratings. The ratings were performed in the same room in which the animals were permanently housed between 0800 and 1100 h (except for those sessions during the time of day study) by raters blind to previous ratings and experimental conditions. High interrater reliability was achieved prior to the study (ICC = 0.966, $p < 0.0001$, based on 29 joint ratings using animals with at least 1 VCM/session).

Exploratory activity in a subgroup of haloperidol-treated female rats chosen randomly was assessed using several measures from a photocell activity monitor (Omnitech model RXYZCM). Variables included distance traveled (cm) and number of vertical body movements (rearing) over six consecutive 10-min epochs. Spearman's correlation coefficient was used to assess the relationship between VCM scores and activity monitor counts from the first and last 10-min epochs as well as total counts for the entire 60 min.

Habituation Studies

The effects of habituation for 2 min, 1 h, and several hours over 4 days were compared to VCM ratings with no habituation (see Table 1). These periods were chosen based on previous studies (5,6,23,28). We also examined the effects of three repeated ratings over 1 day, a rating schedule useful for acute pharmacological challenge studies. Effects of habituation on VCM scores from rats in restraining tubes and their relationship to scores from open cages was investigated over 7 days. Finally, the correlation between general motor activity and VCM scores was examined in haloperidol-treated animals.

Experiment 1: Habituation for 1 hour. Forty female SD rats were randomly assigned to one of two groups, female habituated (F_{hab}) and female nonhabituated (F_{nonhab}), each containing an equal number of haloperidol- and vehicle-treated animals (n = 15 haloperidol; n = 5 vehicle). Rats were individually placed into observation cages, with no prior habituation, and rated for 5 min. Scores were also subdivided for the first 2 min of each 5-min rating period. The 20 F_{nonhab} rats were immediately returned to their home cages where they remained for 1 h. The 20 F_{hab} rats remained in observation cages. After 1 h, the scoring procedure was repeated for all 40 rats. To see if habituation affected VCMs, 5-min ratings for both groups were compared using a repeated measures ANOVA (rmANOVA). To see whether variances for initial and final ratings were related, correlations within groups were examined using Spearman's correlation coefficient. To assess how closely initial and final ratings were matched, the intraclass correlation coefficient (ICC)(1) was used.

Experiment 2: Repeated habituation over several days. Thirty male rats (n = 6 vehicle; n = 24 haloperidol treated) were randomly selected and assigned to one of two groups, male habituated (M_{hab}) or male nonhabituated (M_{nonhab}), each containing an equal number of haloperidol and vehicle treated animals. On day 1, rats were placed into observation cages, immediately rated, and returned to their home cages. VCM scores were recorded during both 2 and 5 min. On days 2 and

TABLE 1
SUMMARY OF EXPERIMENTS

Experiment #	Subjects* (Gender)	Duration of VCM Rating Session	Number of Animals (Vehicle/Hal Rx)†	Comparisons
1	F	2 & 5 min	10/30	Habituation for 1 h vs. no habituation
2	M	2 & 5 min	6/24	Habituation for ~2.5 h/4 days vs. no habituation
3	F	2 & 5 min	10/30	2 min habituation vs. no habituation
4	M	2 min	12/53	Ratings at 3 times of day: 0730, 1130, and 1530 h
5	F	2 min	0/12	Ratings in restraint tube × 15′/day × 6 day vs. no restraint
6	F	5 min	0/56	Correlation of VCMs and locomotor activity

*Male (M) and female (F) groups treated chronically with †vehicle or haloperidol (hal) as described under the Method section.

3, M_{hab} rats were again rated immediately after being placed in the observation cage, habituated for 1 h; and then rated a second time, after which they were returned to their home cages. On day 4, all 30 rats were rated without habituation.

Over the 4-day period, M_{hab} rats spent a total of roughly 2.5 h in the rating cages and were rated six times. Rats in the M_{nonhab} rats spent 10 min in rating cages and were rated twice. To see if habituation affected VCMs, scores from day 1 and 4 for both groups were compared using a rmANOVA. Relationship of ratings within groups was examined using Spearman's correlation coefficient and the ICC.

Experiment 3: Habituation for 2 minutes. Initial, nonhabituated ratings from Experiment 1 (female rats) were used to assess the effects of a brief 2-min habituation period. The number of VCMs/min during the first 2 min was compared to VCMs/min over the next 3 min (of each 5-min rating period) to determine whether the VCMs occurred at a regular frequency through the two rating epochs. Similar comparisons were planned for data from Experiment 2 (male rats). Spearman's correlation coefficient and ICC were used to examine the relationship between ratings.

To explore a related issue, the effect of duration of rating sessions, frequency of VCM scores from 2-min rating epochs were compared to those from 5-min epochs using Spearman's correlation coefficient.

Experiment 4: Repeated ratings over 1 day. One week after Experiment 2, all 65 male rats were rated for 2 min three times over the course of a single day, between 0730–0900 h, 1130–1300 h, and, finally, from 1530–1700 h. Raters were blind to previous treatment and ratings. No habituation period was used. The effect of repeated rating was assessed using a rmANOVA followed by paired *t*-tests, Spearman's correlation coefficient, and the ICC.

Experiment 5: Effects of restraint. Two weeks after Experiment 1, 12 haloperidol-treated female SD rats were randomly assigned to one of two groups, female restrained (F_{restr}) and female unrestrained ($F_{unrestr}$) ($n = 6$). All 12 rats were initially rated for 2 min. The $F_{unrestr}$ group was immediately returned to the home cage. The F_{restr} rats were taken directly from observation cages and allowed to crawl into a cylindrical, clear, Plexiglas tube 3 inches in diameter and 8 inches in length. This limited movement but was not snug. Animals could wriggle from side to side and backwards and forwards. The tube was secured inside a Plexiglas cage, which was placed on the rating platform permitting a clear view of the mouth at all times. Animals' heads did not protrude outside of the tube, as in some prior studies (3,11,12,19,20). On day 1, no ratings were performed in the tubes. Animals spent a total of 15 min in the tubes, exiting for short periods when signs of distress were observed. On days 2 through 6, the F_{restr} rats were individually removed from their home cages, rated for 2 min in observation cages, and placed in the tubes for 15 min each day. At the end of each habituation period, VCMs were again assessed for 2 min while rats were still in the tubes. On the day 7, all 12 rats were rated for 2 min in the open observation cage and immediately returned to their home cages.

Effects of the restraining procedure was assessed in several ways. First, daily scores for the F_{restr} group in cages and tubes were compared using a rmANOVA. Relationships between daily scores for the F_{restr} group were initially examined using Spearman's correlation coefficient. Where rho values are significant, ICC values are also reported. Second, F_{restr} and $F_{unrestr}$ groups were compared using VCM scores from observation cages on days 1 and 7 in a rmANOVA. Relationship between these ratings was assessed separately in the F_{restr} and $F_{unrestr}$ groups using Spearman's correlation coefficient and the ICC.

Experiment 6: Correlation between motor activity and VCMs. To assess the relationship between VCM scores and locomotor activity, all 56 haloperidol-treated female rats were individually placed in activity monitors for 1 h between 0730 and 1100 h. Immediately before and after activity ratings, VCMs were counted for 5 min in observation cages. No VCM ratings had been performed for at least 1 week prior to this study. All animals were naive to the activity monitors. Total and vertical movement scores for the first and last 10-min epochs and for the entire hour were correlated with VCM scores before and after activity ratings.

RESULTS

Male and female rats treated with haloperidol showed a significant, delayed increase in VCMs compared to vehicle treated rats (Fig. 1).

Habituation for 2 Minutes

VCM counts per minute for the first 2 min in observation cages were no different than those for the last 3 min in Experiment 1. These measures were closely correlated (see Table 2). Similar results were seen in Experiment 2 comparing frequencies for the first 2 and the last 3 min (day 1). Post hoc analysis also gave similar results for day 4 (Experiment 2). Correlations of the 2 VCM frequencies for the first 2- and last 3-min epochs were significant both on days 1 and 4 (see Table 2).

The frequency of VCMs recorded during the 2-min and 5-min epochs of both rating sessions during Experiment 1 were highly correlated [$r = 0.9$, $p = 0.0001$, ICC = 0.77, F(39,

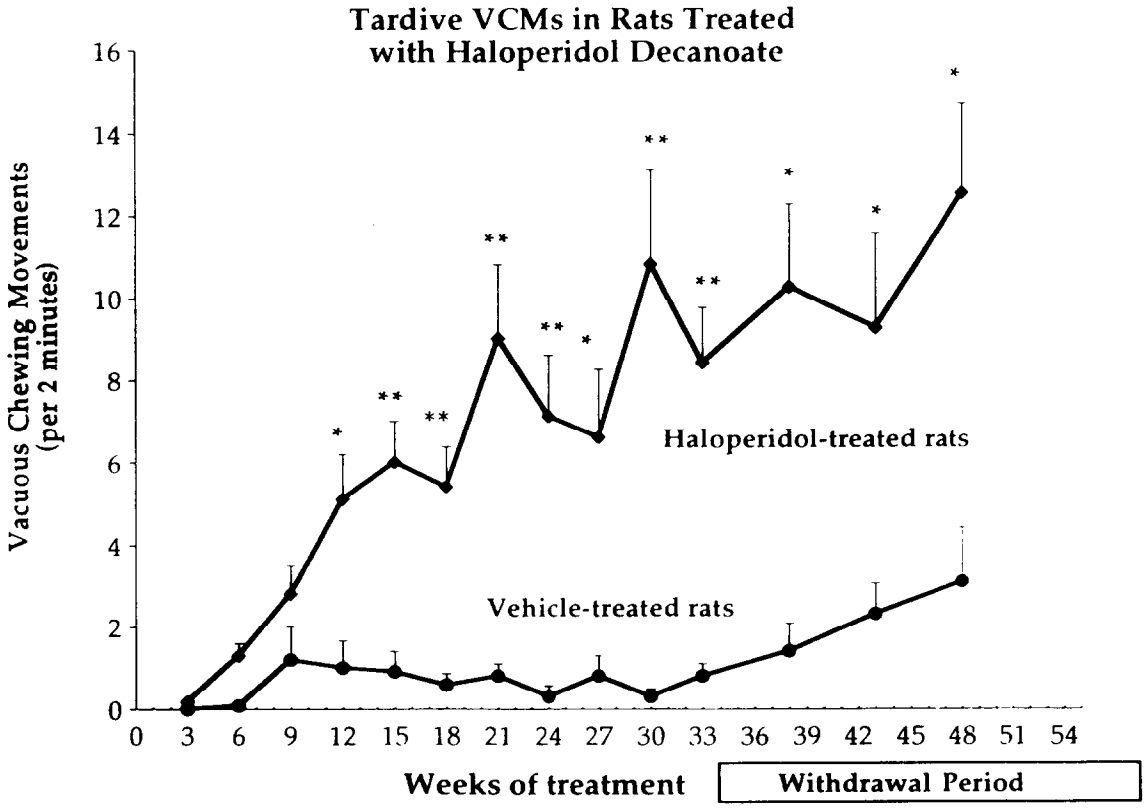

FIG. 1. Delayed onset of VCMs in male rats treated with haloperidol decanoate for 8 months and withdrawn for 5 months. A significant effect is seen for treatment, $F(1, 64) = 22.86$, $p = 0.0001$, and time, $F(12) = 15.5$, $p = 0.0001$. Female rats showed similar treatment and time effects (data not shown). $^{*}p < 0.05$, $^{**}p < 0.001$ compared to vehicle-treated group.

40) = 7.6, $p < 0.001$; $r = 0.83$, $p = 0.0001$, ICC = 0.78, $F(39, 40) = 8.1$, $p < 0.001$ for the initial and final rating sessions, respectively]. Similar analyses with ratings from Experiment 2 also showed a high correlation for each day ($r = 0.80$ to 0.88, $p < 0.005$), suggesting that the length of the rating session, with or without a prior habituation period, does not play a significant role in determining the severity of the VCM ratings.

Habituation for 1 Hour

After 1 h, VCM ratings decreased 11% and 13% for the F_{hab} and F_{nonhab} groups, respectively (Experiment 1) (time ef-

TABLE 2

FREQUENCIES OF VCMs (±SE) IN THE FIRST 2 AND LAST 3 MIN OF 5 MIN RATING SESSIONS FOR FEMALE AND MALE RATS IN EXPERIMENTS 1 AND 2

		VCM Frequency (VCMs/min)		Comparisons (2 vs. 3 Min Frequencies)		
Group	*n*	First 2 Min	Last 3 Min	*p**	Rho (p)	ICC(p)
Females	40	4.76 ± 0.88	5.24 ± 0.78	0.31	0.7 (0.0001)	0.59(0.001)
Males						
Day 1	30	3.45 ± 1.34	3.54 ± 0.01	0.89	0.73 (0.0002)	0.80 (0.001)
Day 4	30	3.68 ± 1.19	3.26 ± 0.97	0.27	0.77 (0.0002)	0.94 (0.001)

*Means are compared with paired *t*-tests. Relationship between individual scores are examined further with Spearmans correlation coefficient and the ICC (*p*-values in parentheses).

fect, $F = 6.2$, $p = 0.02$). There was no significant effect of habituation. Identical results were obtained when vehicle-treated animals were excluded from the analysis. Initial and final ratings were highly correlated for both groups (see Fig. 2) with or without vehicle treated animals.

Repeated Ratings Over 1 Day

In Experiment 4, VCM ratings decreased slightly at the later two time points (Fig. 3). The average ratings (±SE) decreased from 12.1 (±1.5) at 0730 to 10.1 (±1.4) at 1130 to 9.9 (±1.5) at 1530 h. Despite the minor reduction at later times in the light cycle, correlations among all three ratings were high (Fig. 3).

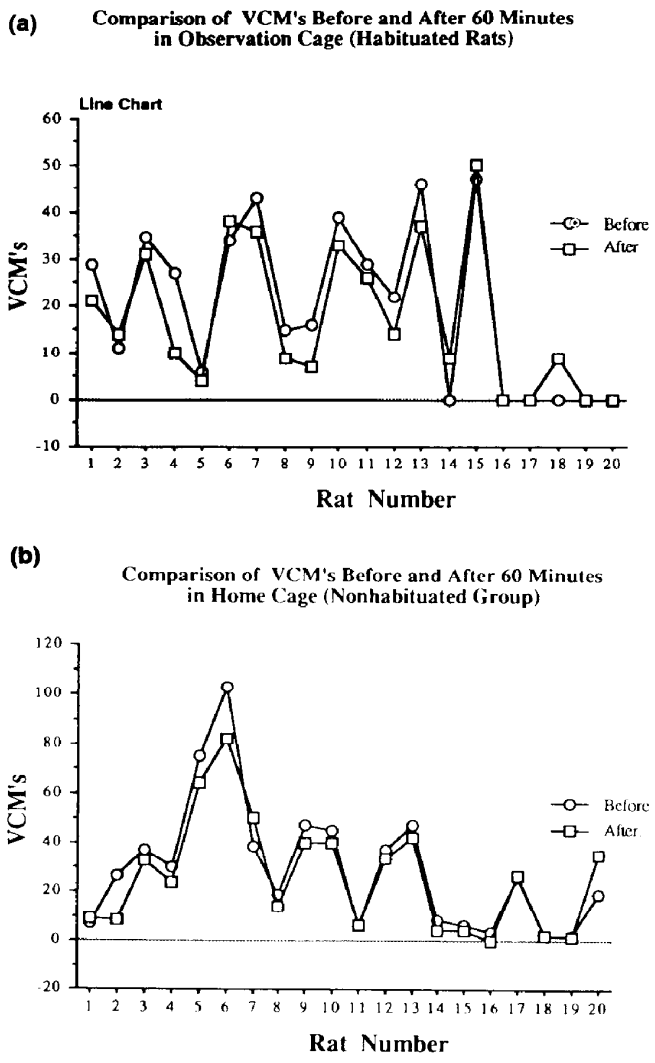

FIG. 2. (a,b) Effects of habituation for 1 h. Comparison of the two VCM ratings (per 5 min) done 1 h apart for (a) F_{hab} and (b) F_{nonhab} rats. There is no effect of group ($F = 1.88$, $p = 0.18$), or time by group interaction ($F = 0.1$, $p = 0.75$), suggesting no habituation effect. Correlations between initial and final ratings: a) F_{hab} group [$r = 0.93$, $p = 0.0001$; $F(19, 20) = 28.0$, ICC = 0.93, $p < 0.001$]; b) F_{nonhab} [$r = 0.95$, $p = 0.0001$; $F(19, 20) = 22.3$, ICC = 0.91, $p < 0.001$]. Correlations were similar when vehicle-treated animals were excluded (e.g., $r = 0.9$, $p = 0.0007$ for F_{hab} and $r = 0.94$, $p = 0.0004$ for F_{nonhab}).

Repeated Habituation over Several Days

Comparing ratings from days 1 and 4 in the M_{hab} and M_{nonhab} groups (Experiment 2), habituation did not affect scores over time (assessed by rmANOVA) (see Fig. 4a-b). Correlations between initial (day 1) and final (day 4) ratings were similar for the M_{nonhab} and M_{hab} groups. When vehicle-treated rats (i.e., VCM scores of 0) were excluded from this analysis, this correlation was reduced in the M_{hab} group only ($r = 0.58$, $p = 0.057$). Pre- and posthabituation ratings from days 2 and 3 were also correlated ($r = 0.86$, $p = 0.001$ and 0.81, $p = 0.002$ respectively). Thus, habituation over several days appeared to have little detectable effect on VCM scores.

Effects of Restraint on VCM Scores

VCM scores in observation cages were not significantly different from those in restraining tubes in the F_{restr} group [rmANOVA, $F(1, 10) = 0.01$, $p = 0.94$]. On the other hand, ratings from open cages were not significantly correlated with those from restraining tubes for any day ($r = 0.26$ to 0.81, $p > 0.05$) except day 6 [$r = 0.99$, $p = 0.03$; ICC = 0.75, $F(5, 6) = 6.9$, $p = 0.02$]. Comparing ratings on days 1 and 7 in the F_{restr} and F_{unrest} cohorts, no group effect was observed, suggesting that repeated use of the restraining tubes did not affect ratings in observation cages (rmANOVA, $F = 0.03$, $p = 0.87$). In support of this, ratings from open cages on days 1 and 7 were highly correlated in the F_{restr} and F_{unrest} groups [$r = 0.93$, $p = 0.04$, ICC = 0.91, $F(5, 6) = 23.0$, $p < 0.001$; $r = 0.98$, $p = 0.03$, ICC = 0.96, $F(5, 6) = 44.9$, $p < 0.001$, respectively].

Activity and VCM Scores

No significant correlations were seen between any behavioral measure assessed in the activity monitor and VCM scores counted before or after animals were placed in the monitors (Experiment 6). This includes total distance traveled in 1 h (Fig. 5), total vertical movements (rearing) in 1 h, and total distance traveled in the first and last 10 min epochs.

DISCUSSION

Habituation procedures, whether for 2 min, 1 h, or several hours over 4 days, had little detectable effect on VCM scores in rats following long-term treatment with haloperidol. This was shown using several types of analyses from several habituation experiments. First, VCMs/min over the first 2 min in observation cages were no different than, and were highly correlated with, VCMs/min over the next 3 min. This suggests that 2-min habituation periods have little detectable effect on assessment of VCM severity. ICC values appeared somewhat higher in male rats compared to female rats. This correlation was slightly higher after several rating sessions, raising the possibility of a slight habituation effect (Table 2). In a related analysis, VCM frequency during rating sessions lasting for 2 and 5 min were highly correlated, suggesting that shorter periods are adequate for VCM assessment. On the other hand, 2-min rating sessions could lead to some loss of resolution in animals with infrequent VCMs. No differences in VCM scores were detectable in rats habituated for 1 h on several consecutive days compared with unhabituated animals. Furthermore, correlation with baseline VCM ratings were highly significant whether rats were or were not habituated to rating cages for 1 h

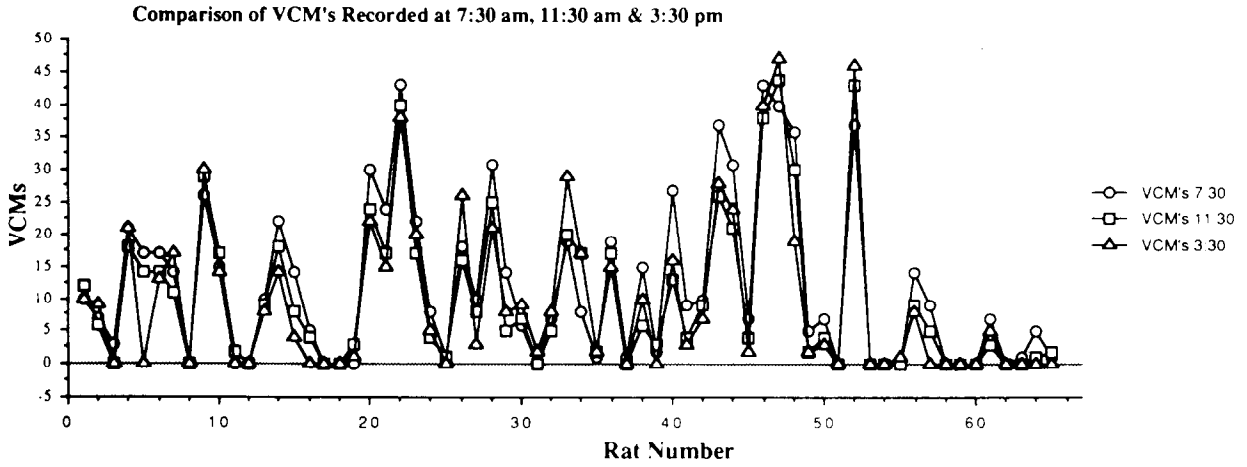

FIG. 3. Repeated ratings over 1 day. VCM scores from 2-min observation periods were recorded at 0730, 1130, and 1530 h. Significant reductions were found at the later time times [rmANOVA, $F(2, 64) = 10.44$, $p = 0.0001$; 0730 vs. 1130, $t = 4.13$, $p = 0.0001$; 0730 vs. 1530, $t = 3.38$, $p = 0.001$]. Despite different means, these scores are highly correlated ($r = 0.89$–0.96, $p < 0.0001$; ICC for all three ratings = 0.93, $F(64, 130) = 39.8$, $p < 0.0001$).

or for several days. Considered together, these data suggest that habituation has little effect on the assessment of VCM scores.

Repeated ratings over 1 d were highly correlated. This was demonstrated by typical correlation coefficients above 0.85 for the three Experiments (1, 2, and 4) using at least two VCM measures in 1 day (see Figs. 2 and 3). Results from three repeated ratings in 1 day indicate that correlations were slightly higher for rating sessions closer together. This suggests that the large variances seen in drug challenge studies could be markedly reduced by comparing ratings before and after treatment, close in time, on the same day.

Previous studies have shown that only a subgroup of treated animals develop the VCM syndrome during long-term neuroleptic administration (2,25,26). Identification of affected rats using repeated ratings over time may facilitate the assessment of responses to drug challenges. For example, one could examine whether a drug could increase movements in animals that do not have the VCM syndrome while suppressing movements in animals that do, such as might occur with increasing doses of haloperidol (2). Alternatively, a drug that worsened VCMs in those with and without the VCM syndrome would suggest that the agent itself produces abnormal chewing movements. This may be the case with cholinergic agonists (24). The results of the current study show that additional habituation procedures, beyond whatever habituation the initial triweekly rating sessions provide, do not affect assessment of VCMs and are not required. It is unclear if habituation prior to the initiation of haloperidol treatment would affect early VCM assessment. The animals used in this study, however, only spent a total of 30 min in the rating cages over a 1-year period. It seems unlikely that such very brief exposure has a major impact on the effect of habituation. Thus, the negative results of the current study on habituation may also apply to early VCM assessment.

Differences in habituation probably do not account for conflicting findings in drug challenge studies with D_2 antagonists (7a,27,28,30) and cholinergic agonists and antagonists (7a,17,22,24). However, data from the restraint experiment raise issues about this method. Most studies that have failed to find increased VCMs during chronic neuroleptic treatment have tested animals in restraining tubes (3,11,12,18a,19,20). Of these, two used both rating methods and found significant increases in VCMs in ratings from open cages but slight or no changes in ratings from restraining tubes (12,19,20). Other studies that have used restraining tubes, however, have found increased VCMs with chronic typical, but not atypical, neuroleptic treatment (6b,7c), and have seen correlations between restrained and unrestrained ratings (6b). Results from the current study suggest that VCMs in tubes become more highly correlated with those in observation cages in haloperidol-treated rats only after long habituation periods. This supports the idea that some other process, such as stress, is impacting the expression of VCMs (6b). It has been argued that VCMs are simply missed in vehicle-treated rats that are actively moving, and that restraints are required to equally assess neuroleptic and vehicle-treated animals. This idea is contradicted by the observation that VCMs in restraints are increased compared to VCMs in cages whether or not rats are cataleptic (12). Thus, it seems likely that restraint may increase VCMs by some mechanism other than simply reducing locomotor activity.

To test the possibility that stress from restraint, rather than reduced locomotor activity, elevates VCM scores in vehicle-treated animals, we examined the effects of restraint using previously described habituation methods (3,11,12,19,20) on 10 rats that had been previously treated with vehicle for 5 months. Rats were rated every other day over 3 weeks first in open cages (for 2 min) then while restrained in tubes (for 6 min) [see (3,11,12,19,20)]. On average, less than 0.5 VCMs/rat was observed in open cages for all rating sessions. During the first restrained rating session, 5.8 ($\pm$1.4) VCMs/rat were seen. By the 10th rating session (after 60 min of habituation over 3 weeks), 26.1 ($\pm$4.2) VCMs/rat were counted. Thus, rather than habituate, these VCMs increased over time (session 1 vs. 10, paired t-test, $t(9) = -5.37$, $p = 0.0005$). This time-dependent increase suggests that use of restraining tubes, rather than simply making observations easier, can actually

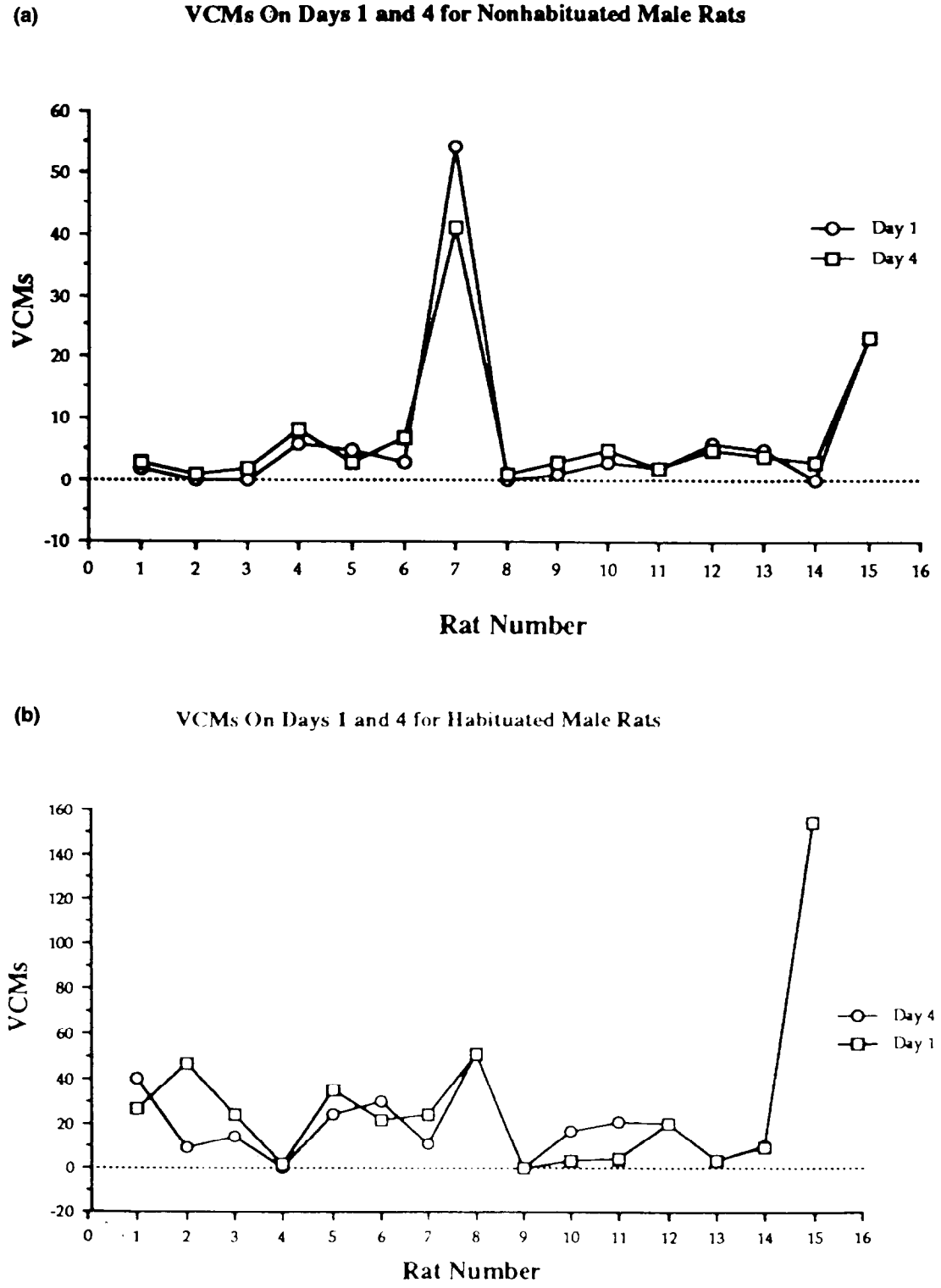

FIG. 4. (a–b) Effects of habituation for several days, demonstrated by VCM scores in male rats from Experiment 2. No significant effects for time, group, or time by group interaction were seen ($F = 0.02$ $p = 0.90$; $F = 3.19$, $p = 0.08$; and $F = 0.35$, $p = 0.56$, respectively). Correlations between initial (day 1) and final (day 4) VCM ratings for each group were: (a) M_{nonhab} rats, $r = 0.64$, $p = 0.02$; ICC = 0.94, $F(14, 15) = 31.8$, $p < 0.001$; (b) M_{hab} rats, $r = 0.69$, $p = 0.01$; ICC = 0.94, $F(14, 15) = 30.5$, $p < 0.001$.

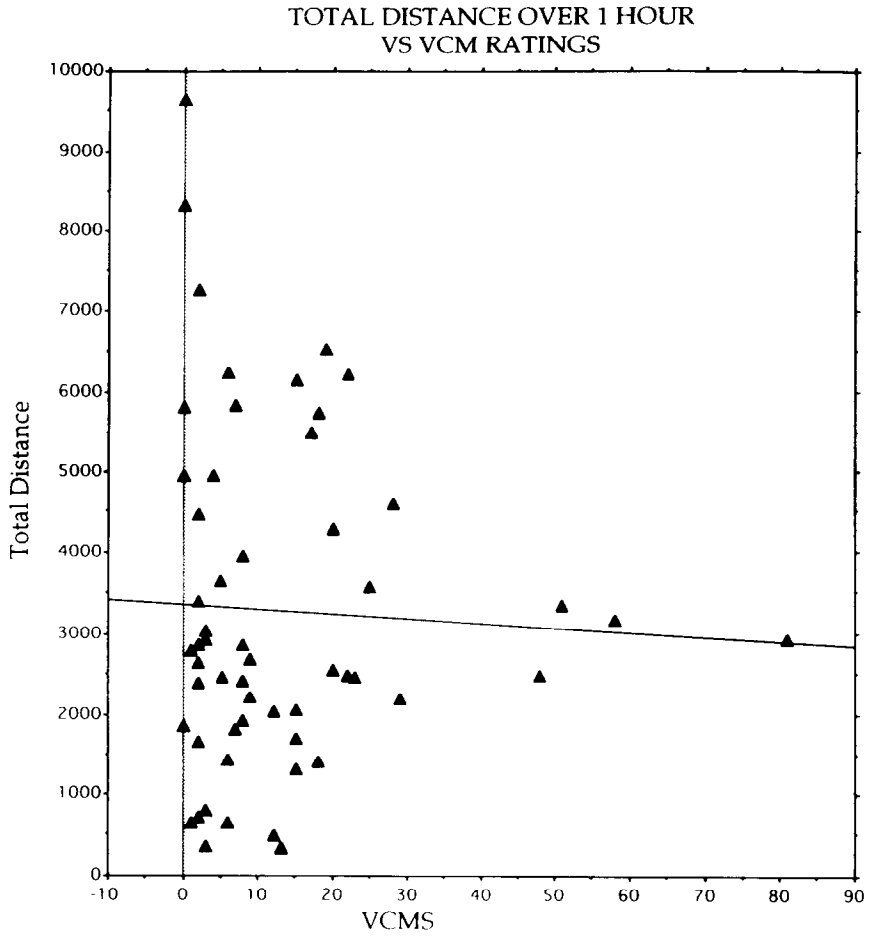

FIG. 5. Correlation between VCM scores (per 5 min) and total distance traveled (cm) in 1 h. No significant correlation was seen for this or any other activity measure.

induce VCMs that are not quickly extinguished by usual habituation procedures. Furthermore, these restraint-induced VCMs observed in vehicle-treated rats, while produced by a different mechanism, are phenomenologically indistinguishable from neuroleptic-induced VCMs. It seems unlikely that the increase we observed over a relatively brief time would continue for more extended periods. Other studies that have recorded VCMs in restraints for long periods have observed no increase (19,20) or a decrease (6b) in vehicle-treated animals. Nevertheless, persistent increased VCMs in control animals may account for failure of some studies to observe a significant increase in tardive VCMs in neuroleptic-treated animals.

We attempted to test further the hypothesis that VCMs compete with locomotor behavior by examining the correlation between locomotor activity and VCM scores in a group of haloperidol-treated female rats. No correlation was seen between VCM scores and any measure of activity. One deficiency with this approach is that activity and VCMs were not measured concurrently or in the same cage. It seems reasonable to assume, however, that individual rats would have similar activity patterns in different cages despite minor physical differences. Furthermore, data from Experiments 1, 2, and 4 show that VCM ratings remain fairly stable over hours and days in a given rat. Thus, the lack of correlation between exploratory activity and VCM scores over the course of 1 h offers support for the notion that VCMs occur independently of reduced motor activity. A prior report of a significant inverse correlation between activity levels and VCM scores included ratings from vehicle and haloperidol-treated animals (19). Including vehicle-treated animals may distort results due to the obvious relationship between haloperidol treatment and catalepsy. It should be noted, however, that in haloperidol-treated animals, a relationship may exist between measures of catalepsy and VCM severity [see (8) for discussion]. Nevertheless, the results from the current study are consistent with the hypothesis that failures to see increased VCMs in studies using restraints might be due to stress-related alterations in oral activity in vehicle-treated animals.

Although the use of restraining tubes may have produced significant alterations in VCMs in some prior studies, this method has permitted a more detailed, quantitative analysis of jaw movements with improved resolution for very small amplitude movements. Such studies have shown that neuroleptics may induce time-dependent changes in the form of oral movements that persist on neuroleptic withdrawal. These

so-called computer scored movelets bear some similarites to motor disturbances reported in TD (19,20). Results from the current study, however, suggest that these reports should be interpreted with caution.

In conclusion, the results of this study suggest that habituation procedures are unnecessary for the reliable assessment of VCMs prior to the initiation of drug challenge studies. Due to the very limited prior exposure of rats to the rating procedures (13 times over 12 months), these results may be applicable to initial VCM ratings as well. Rating sessions of 2 min appear adequate, giving results that are highly correlated with 5-min ratings. Time of day may affect ratings, with slightly lower scores occurring later in the light cycle. Neuroleptic-induced VCMs from restrained rats are correlated with VCM ratings from open cages only after several hours of habituation. No habituation is seen in vehicle-treated rats rated in restraining tubes. This suggests that stress from restraint may alter expression of oral activity. Finally, the lack of correlation between exploratory activity and VCMs argues against the hypothesis that VCMs are simply due to reduced motor activity. Thus, habituation and forced restraint do not appear to be necessary to produce reliable measures of VCMs.

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