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Factor analysis of open-field behavior in the rat (*Rattus norvegicus*): application of the three-way PARAFAC model to a longitudinal data set

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Abstract

We examined the multivariate nature of open-field behavior in adult male rats ($n = 26$) by subjecting a longitudinal data set, obtained during 4 open-field test sessions (4 min in duration and spaced 48 h apart), to a three-way PARAFAC analysis. Unlike conventional two-way factor analytical models, the PARAFAC procedure allows for the direct factor analysis of 3-dimensional arrays, which then provided a *unique* factor solution to the longitudinal data set. The PARAFAC analysis extracted 2 factors: i) emotional reactivity and ii) exploratory behavior. These two factors changed in temporal prominence, with animals showing greater emotional reactivity on the first test session, and greater levels of exploration on the third and fourth test sessions. These results are in general agreement with previous findings which used more conventional factor analytic approaches. These findings indicate that multivariate procedures, such as the PARAFAC analysis, can be helpful in the quantitative characterization of behavioral phenomena in a more 'realistic' manner.

Key words: Emotional reactivity; Exploratory behavior; PARAFAC analysis; Factor analysis; Temporal changes; Open-field; Rat

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Introduction

Since its development by Hall (Hall, 1934, 1936; Hall and Ballechey, 1932), the open-field test has become one of the most widely used instruments in animal behavior analysis (see reviews by Archer, 1973; Walsh and Cummins, 1976). Typically, the open-field apparatus consists of a novel open space from which escape is prevented by a surrounding wall. An animal, usually a rodent such as a rat or mouse, is placed in this apparatus for some fixed time interval and the incidence and/or duration of certain behaviors are recorded. Well over 20 different behaviors have been quantified in the open-field test, although only a few have been shown to be reliable for the rat (cf. Walsh and Cummins, 1976). Ambulation (number of subdivisions entered), rearing response frequency, and defecation responses (number of fecal boli), have been shown to be reliable measures (Ivinskis, 1968). These measures also tend to be the most common variables used. Recently, it was shown that by aggregating scores over test sessions, reliability could be increased substantially for these measures (Ossenkopp and Mazmanian, 1985b).

Interpretation of the behavioral measures obtained in open-field testing has been problematic. Most measures of motor behavior have been viewed as indices of arousal or exploration, and the defecation measure has been used as an index of emotionality or autonomic reactivity (Broadhurst and Eysenck, 1964; Ivinskis, 1970; Russell et al., 1987; Vanderwolf et al., 1988; Walsh and Cummins, 1976).

Recognition of the fact that 'single variable' approaches to the study of behavior are often inadequate to describe a behavioral phenomenon (e.g., Hinde, 1970; Ossenkopp and Mazmanian, 1985a; Whishaw et al., 1983) has led to the suggestion that behavioral profiles might be preferable (see Robbins, 1977). However, there are problems associated with studying several variables independently, since their interrelationships are not taken into account (Frey and Pimentel, 1978). Indeed, correlations between the major open-field variables of ambulation, rearing, and defecation have been examined frequently (e.g. Anderson, 1938; Archer, 1973; Broadhurst, 1957; Holland and Gupta, 1966; Ossenkopp and Mazmanian, 1985b; Pare, 1964; Tachibana, 1982; Walsh and Cummins, 1976) and large positive correlations often have been obtained for the two activity variables. In contrast, defecation scores tend to correlate negatively with both ambulation and rearing. A variety of multivariate analytical methods, such as principal component and factor analysis, are designed to deal with multivariate measures, and these methods tend to promote 'phenomenon realism' by more adequate characterization of general behavioral processes (cf. Ossenkopp and Mazmanian, 1985a).

Factor analysis is used for the reduction or simplification of data (e.g. Cattell, 1952; Nunally, 1978) and allows for the characterization of a large number of variables or observed relationships in terms of a smaller set of hypothetical or higher order variables. Factor analysis reduces the original set of variables to a smaller, more complex and abstract set of variables, called factors, which acquire 'meaning' because of the structural properties that may exist within the set of relationships (Ferguson, 1981; Harris, 1975). Essentially, a larger set of variables is subjectively reduced to a smaller set, which then gives a broader interpretation to a particular behavioral process.

Relatively few studies have employed factor analytical methods to examine open-field behaviors (see Royce, 1977; Walsh and Cummins, 1976). Whimbey and Denenberg (1967a,b) provided the first evidence for factors which they labelled 'emotional reactivity' and 'exploration'. Defecation frequency loaded on the emotionality factor and ambulation

scores loaded on both factors. Ambulation on the first test day correlated positively with defecation scores, and had a positive loading on the emotionality factor. On subsequent days ambulation correlated negatively with defecation scores and had a large negative loading on the emotionality factor. All ambulation scores had positive loadings on the exploration factor. Subsequent research has generally obtained similar factors in open-field behavior (e.g. Maier et al., 1988; Ossenkopp and Mazmanian, 1985c; Royce, 1977).

Although most researchers recognize the importance of examining open-field behavior over several test sessions in order to gain information about changes in the variables with repeated testing, these data have been difficult to deal with statistically. Conventional factor analytical procedures typically use a subject by variable, or variable by variable, two-way design (Frey and Pimentel, 1978; Harris, 1975). When longitudinal data sets are examined, the additional dimension presents a problem in terms of the appropriate analytical approach. One solution has been to avoid the third dimension by aggregating the variable over trials or days (e.g. Maier et al., 1988; Ossenkopp and Mazmanian, 1985c), thus, losing the additional information contained in the longitudinal dimension. Another approach is to divide the three-way data set into a series of two-way slices and then perform separate factor analyses on each slice. However, there are several different ways to 'slice' a three-way array and this complicates interpretation (see Cattell, 1952). Recently, several new approaches to factor and principal component analysis have been developed which can directly analyze such three-dimensional arrays (e.g. Harshman, 1970; Harshman and Lundy, 1984). In the conventional two-way factor analysis many alternative rotations will produce factors that fit the data equally well. In the PARAFAC procedure, developed by Harshman (1970), one 'rotation' will fit the rotation across time better than any other, and thus, the PARAFAC analysis provides an empirically based procedure for selecting the best candidates for 'real' factors within a given domain. The PARAFAC three-way model is based on Cattell's (1944) idea of 'parallel proportional profiles'. It describes the relationship between the factor loadings on two different occasions in which the factors change their relative influence in a proportional manner (Harshman and Lundy, 1984). That is, a factor should retain its pattern of effect from one occasion to the next. Furthermore, there must be systematic factor differences across the third dimension (e.g. occasions) that are not simply reflecting error due to random sampling, and each factor must have a pattern of effect in each mode that is distinct from all other factors (Harshman and Lundy, 1984).

To gain more insight into the factor structure of open-field behavior in rats, and to demonstrate the application of the PARAFAC procedure, we examined a longitudinal data set obtained during four test sessions spaced 48 h apart. Eleven variables, obtained for 26 rats during each of these 4 test sessions, were subjected to the PARAFAC analysis. The results were then compared to those obtained with more traditional two-way analyses.

Materials and Methods

Subjects

Twenty-six adult, male, hooded rats (Long-Evans strain, Charles River, Quebec) were used as subjects. The animals weighed between 200 and 230 g at the start of the experiment, and were individually housed in stainless steel wire mesh cages. The colony room was maintained at $22 \pm 1^\circ\text{C}$ and on a 12 h light: 12 h dark cycle with lights on from

0700 to 1900 h. The rats were given continuous access to food (Purina pellets) and tap water.

Open-field apparatus

The test apparatus was a circular open-field similar to the one used by Broadhurst (1957). It was 90 cm in diameter with a 30 cm high wall. The floor and the wall of the open-field were black, and the floor was divided into 25 equal area sections by thin white lines. These lines consisted of 3 concentric circles, with diameters of 18 cm for the inner circle, 54 cm for the next circle, and 90 cm for the third circle, respectively. The smaller annulus was divided into 8 equal segments and the larger annulus into 16 equal segments. A transparent plastic coating covered the entire floor area. The open-field was located inside a large wooden frame which was surrounded on all sides by black curtains. The field floor was illuminated by two 60 W fluorescent lights located 100 cm above the floor. A white-noise generator provided a masking noise of 61 ± 1 db (measured with a Bruel & Kjaer sound level meter, type 2203) at the floor of the open-field apparatus.

Behavioral procedure

Four 4-min open-field tests were administered to each animal at 48 h intervals. At the start of each session the rat was placed in one of the peripheral sections in the outer annulus of the open-field (next to the wall) and the animal's behavior was monitored by an experienced observer for 4 consecutive minutes. The following variables were recorded for each minute of each test session: 1) ambulation – the number of open-field sections entered by all 4 feet of the animal; 2) rearing responses – the number of times the animal raised both forefeet off the floor and extended its body; 3) defecation – the number of fecal boli deposited; 4) urination – frequency of urination; and 5) grooming – frequency of grooming bouts. The observer stood outside the black curtains surrounding the apparatus to avoid influencing the animals' behavior during the recording period. At the end of each session the animal was returned to the home cage and the floor of the open-field was cleaned and sponged over with a weak vinegar solution to remove any residual odors.

Data reduction and analysis

The following 11 variables were incorporated into the analysis: 1) ambulation, 2) defecation, 3) rearing, 4) urination, 5) grooming, 6) negative thigmotaxis, 7) latency to defecate, 8) latency to enter the inner circles, 9) ambulation habituation, 10) rearing habituation, and 11) inner circle activity (number of sections entered in the inner two circles). 'Negative thigmotaxis' was a ratio measure defined as activity in the inner two circles divided by total activity (e.g. Sanberg and Ossenkopp, 1977). Ambulation and rearing habituation measures were calculated as the signed difference scores between the fourth and the first minute ambulation and rearing totals, respectively, of a test session. As these behavioral measures were not in commensurate units, the data were preprocessed to remove scale differences, by standardizing behavioral variables and days, to unit variance. The measures were then assembled into a single 11 (variables: Mode A) by 4 (days: Mode

B) by 26 (subjects: Mode C) matrix for analysis. Thus, there were a total of 104 (4 days \times 26 subjects) replicates of each variable, providing a ratio of about 1 : 10 of variables to replicates.

Results

Data analyses were conducted twice; once with the requirement that the factors for Mode A (variables) be orthogonal, and once with the same requirement for Mode B (days). Since subjects were chosen randomly, orthogonality was not imposed on Mode C. In all cases three independent solutions were derived as the number of factors was incremented from one through four. To check the stability of the solutions, correlations were calculated among respective factors for each of the three solutions. For the first three factors, the correlations between corresponding solutions were near perfect. This correspondence broke down at four factors, giving the first indication that too many factors had been derived from the data set. Given this high degree of correspondence between solutions, only the first solution is detailed in the following sections.

Orthogonality constraint on Mode A

To confirm that three was the optimal number of factors for this data set, three additional indicators were examined — two ways of measuring the fit between the original data and that predicted by the factor solution, and the cross-products among the factors (see Harshman, 1984). The square of the correlation between the derived matrix and the raw data matrix is an estimate of the variance accounted for. When plotted, this curve will

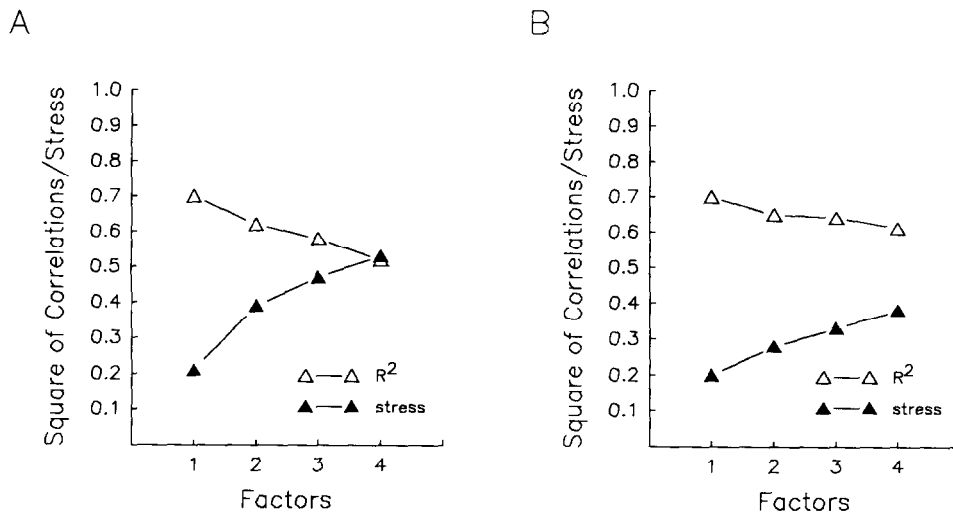


Fig. 1. Squares of the correlations between the derived matrix and the raw data matrix (open triangles), and the 'stress' measure (filled triangles) as a function of the number of derived factors. Left panel: orthogonality for Mode A (variables); Right panel: orthogonality for Mode B (days).

increase monotonically and, at some point, there will be an abrupt transition to a smooth, probably linear, curve which increases very slowly. These final small improvements in fit likely are due to fitting 'noise' in that data. The 'elbow' that occurs at this point, suggests that additional factors are fitting error variance. The second measure of fit is an analog of least squares regression that minimizes the residual sum of squares. This goodness-of-fit measure, called 'stress', was described by Kruskal and Wish (1968). Both of these 'fit' measures are plotted in the left panel of Fig. 1, and each suggests a two- or three-factor solution. The third way of assessing dimensionality is to examine the correlations and cross-products among the factors at each dimensionality. Large values on each measure suggest that too many factors have been derived. The pattern of relationships among the

TABLE 1

Cross-products of normalized factors (i.e. cosines of the angles between the factors) for the two-, three-, and four-factor analyses with an orthogonality constraint on Mode A (variables). Note that Mode B represents days and Mode C subjects

	Factors			Factors		
	1	2		1	2	3
Mode A						
1	1.000	0.000	1	1.000	0.000	0.000
2	0.000	1.000	2	0.000	1.000	0.000
			3	0.000	0.000	1.000
Mode B						
1	1.000	0.958	1	1.000	0.950	-0.923
2	0.958	1.000	2	0.950	1.000	-0.883
			3	-0.923	-0.883	1.000
Mode C						
1	1.000	0.688	1	1.000	0.645	0.706
2	0.688	1.000	2	0.645	1.000	0.678
			3	0.706	0.678	1.000
	Factors					
	1	2	3	4		
Mode A						
1	1.000	0.000	0.000	0.000		
2	0.000	1.000	0.000	0.000		
3	0.000	0.000	1.000	0.000		
4	0.000	0.000	0.000	1.000		
Mode B						
1	1.000	0.907	0.994	-0.877		
2	0.907	1.000	0.906	-0.809		
3	0.994	0.906	1.000	-0.883		
4	-0.877	-0.809	-0.883	1.000		
Mode C						
1	1.000	0.779	0.594	0.426		
2	0.779	1.000	0.321	0.566		
3	0.594	0.321	1.000	0.175		
4	0.426	0.566	0.175	1.000		

factors was the same for both the correlations and the cross-products. The cross-product values are shown in Table I. The cross-products on Mode A are zero due to the orthogonality constraint on this aspect of the data. The cross-products among all the factors were relatively high, but with the fit-dimensionality curves suggesting a two-factor solution.

To confirm this, and to aid in interpretation, plots of the factor loadings for the two- and three-factor solutions are shown in Figs. 2 and 3. In each of these figures the solid line indicates a zero loading with positive values above this line, and negative ones below it.

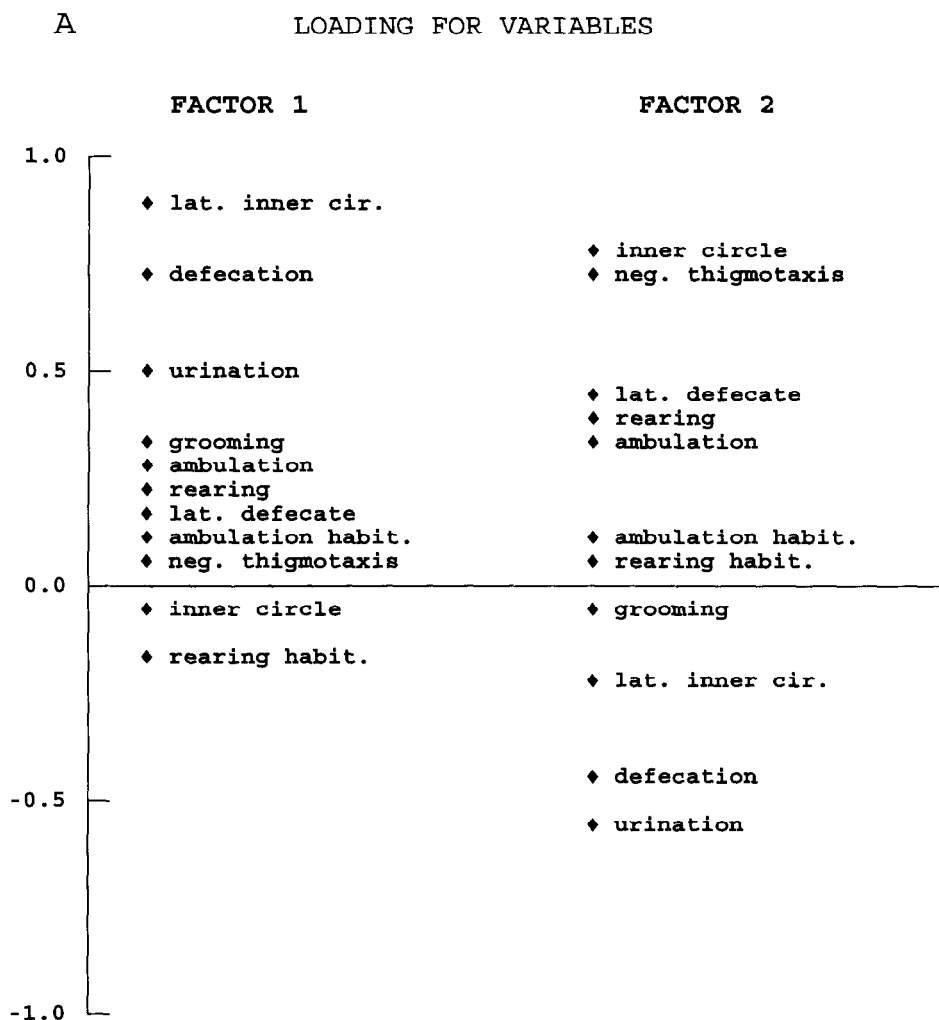


Fig. 2. Factor loadings for the two-factor solution with orthogonality on Mode A (variables). Panel A: loadings for variables; Panel B (over): loadings for days. Note that values above the zero line are positive and values below this line are negative.

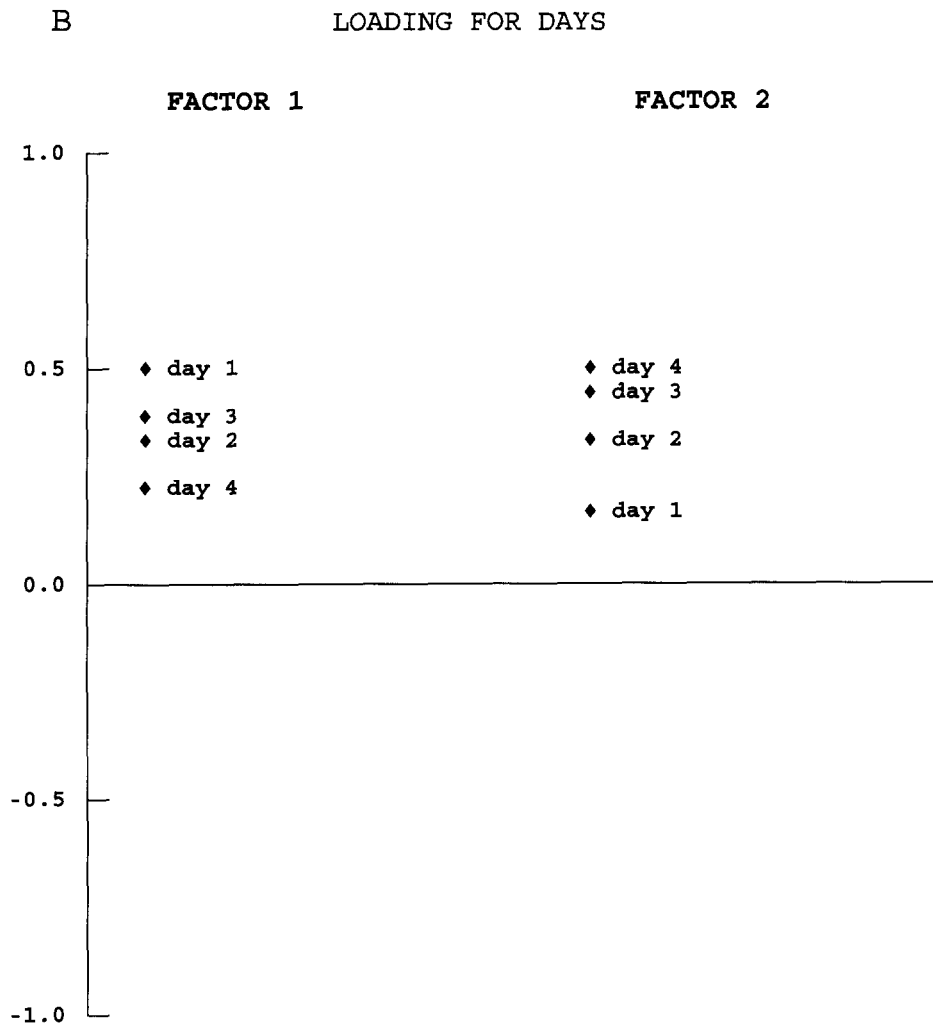


Fig. 2 (continued).

Although the Mode A and B loadings are plotted together, their reference scales are not identical. The two-factor solution is readily interpretable. Latency to enter the inner circles and defecation levels were the variables that loaded highest on Factor 1, while inner circle activity and negative thigmotaxis loaded highest on Factor 2. Day 1 loaded highest on Factor 1, suggesting that latency to the inner circles and defecation were important variables on that day. Similarly, Day 4 loaded highest on Factor 2, with inner circle activity and negative thigmotaxis, suggesting an important role for these variables on that day. When a third factor was extracted, these first two factors remained essentially the same. The third factor (Fig. 3) exhibited a negative relationship between the habituation variables and the days aspect of the data.

Orthogonality constraint on Mode B

In an effort to further understand the days aspect of the data, the full series of analyses were repeated with the orthogonality constraint imposed on Mode B. As in the previous analyses, one through four factors were extracted and the fit-dimensionality curves are shown in the right panel of Fig. 1. These curves again suggested a two-factor solution. The three solutions obtained for the two-factor analysis were compared, and perfect convergence was obtained. The cross-products of the factors are presented in Table 2 and a plot

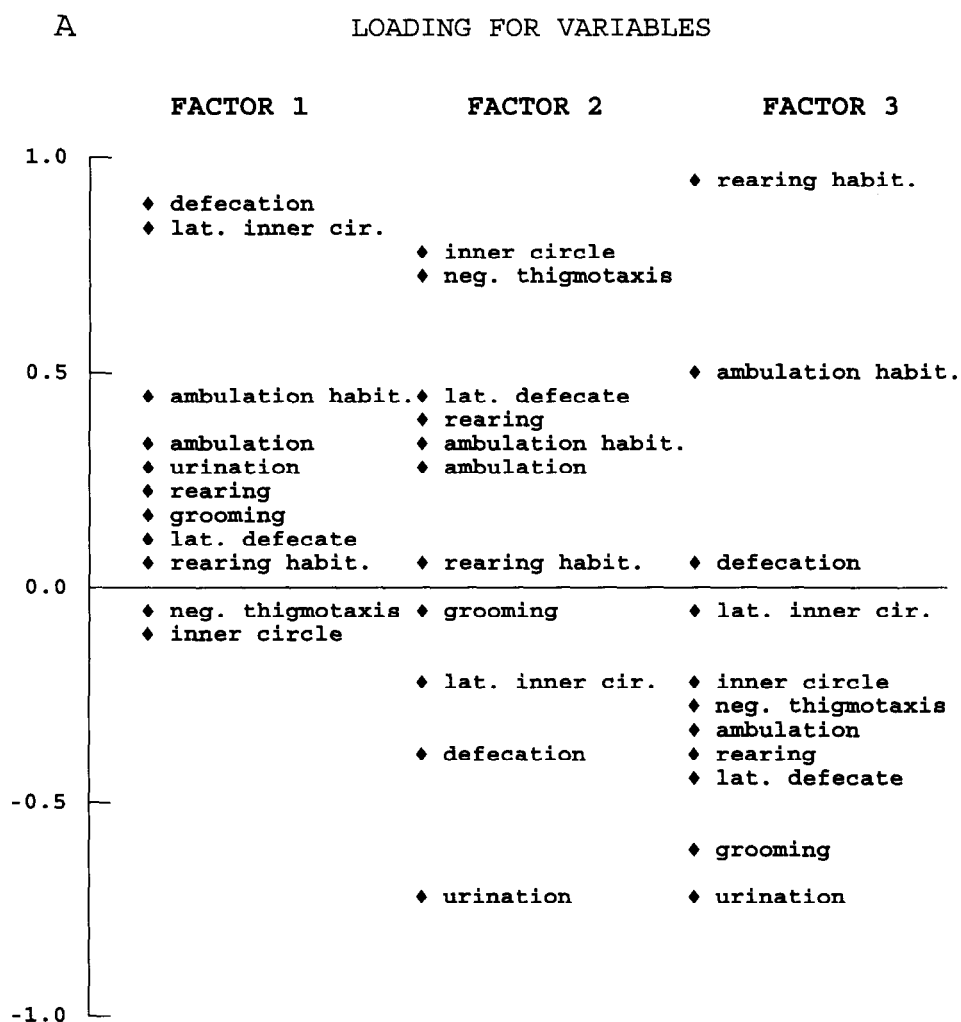


Fig. 3. Factor loadings for the three-factor solution with orthogonality on Mode A (variables). Panel A: loadings for variables; Panel B (over): loadings for days. Note that values above the zero line are positive and values below this line are negative.

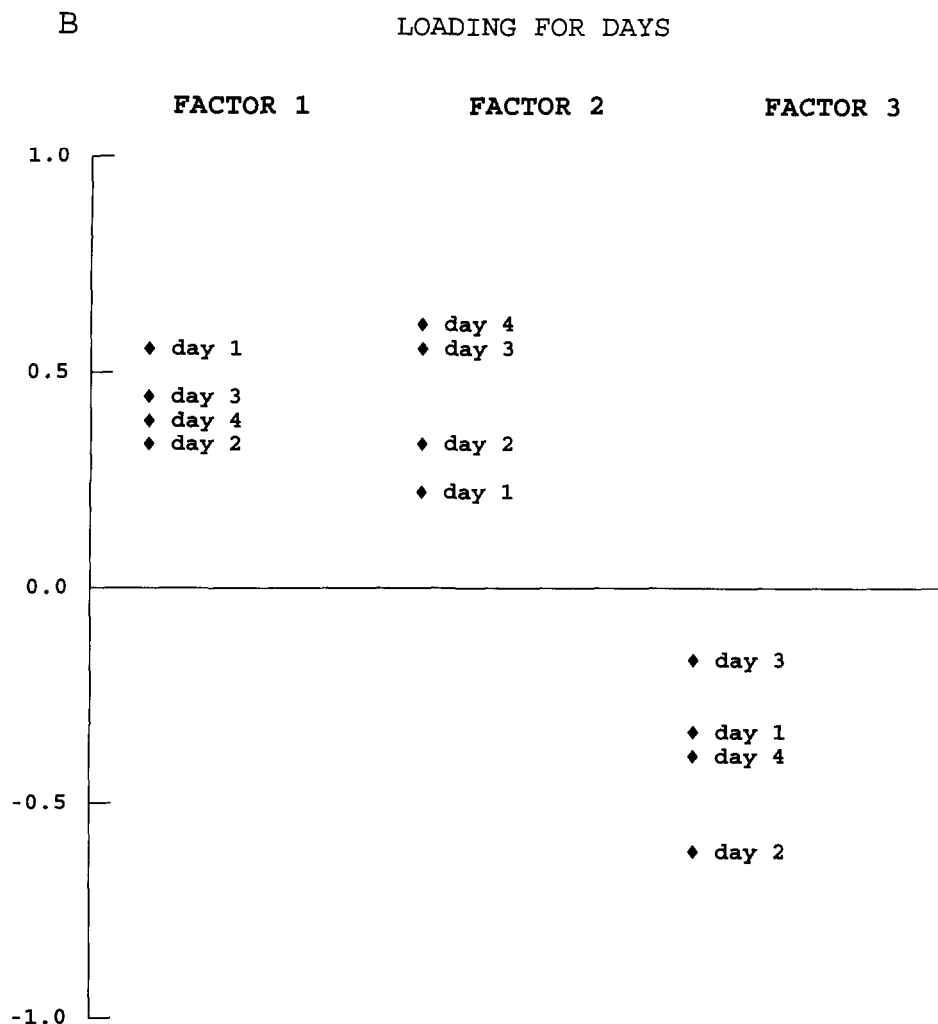


Fig. 3 (continued).

of the factor loadings is shown in Fig. 4. The general similarities between the solutions can be seen by comparison of Figs. 2 and 4. The negative poles on corresponding factors are nearly identical. The relative position of the days mode is also similar, with Day 1 loading highest on Factor 1 and Day 4 highest on Factor 2. The greatest differences occur between the variables on the positive poles of the factors. Here ambulation, rearing, and latency to defecate, have their greatest loadings on Factor 1, while habituation in rearing, along with inner circle activity, and negative thigmotaxis, show the most loading on Factor 2.

In summary, it appears that the two-factor solution for this three-way data set meets the requirements of convergence, stability, and interpretability. Day 1 seems to be distinctly different from Days 3 and 4, with variables, such as latency to enter the inner circles,

defecation, and urination, playing an important role on Day 1, but not on Days 3 and 4. Inner circle activity and negative thigmotaxis are the important variables on these later days of the open-field test. These relationships are relatively consistent, despite changes in orthogonality constraints in the analyses.

Discussion

Application of the three-way PARAFAC procedure has the advantage of reducing the subjectivity of the analysis, by avoiding the problems of a number of possible data 'slices',

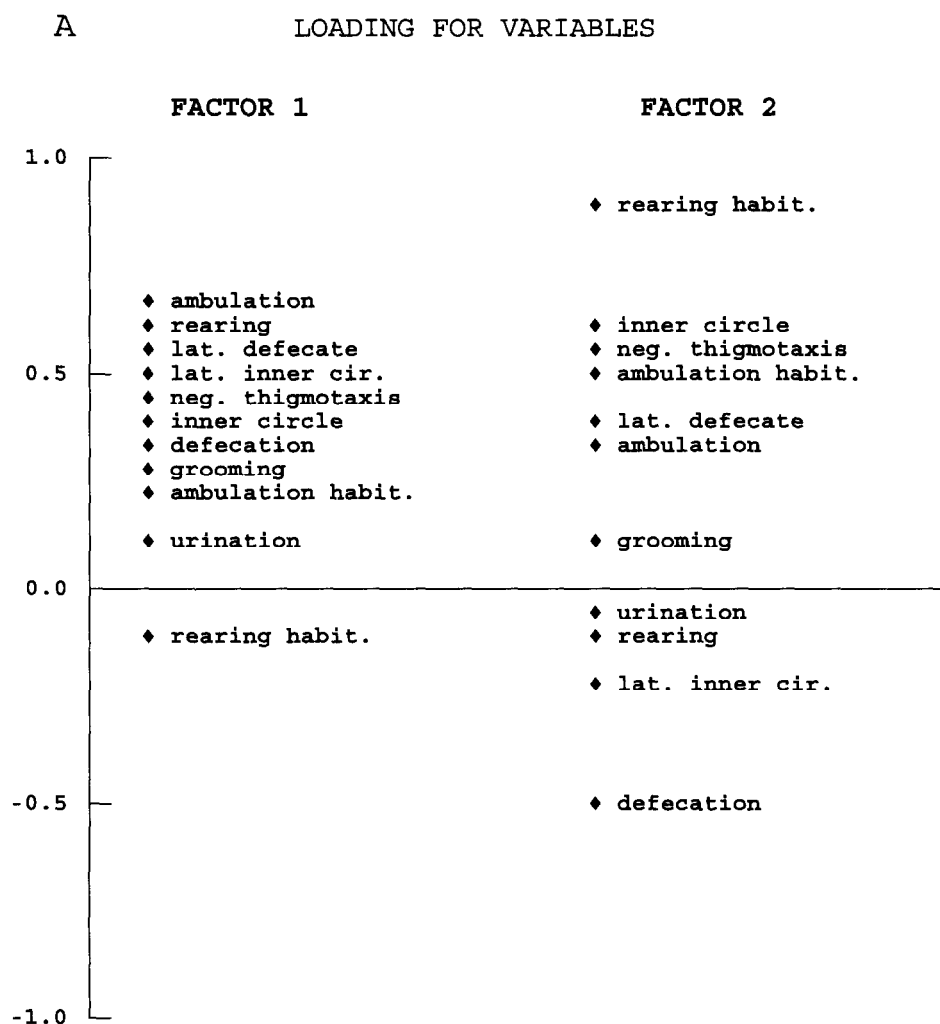


Fig. 4. Factor loadings for the two-factor solution with orthogonality on Mode B (days). Panel A: loadings for variables; Panel B (over): loadings for days. Note that values above the zero line are positive and values below this line are negative.

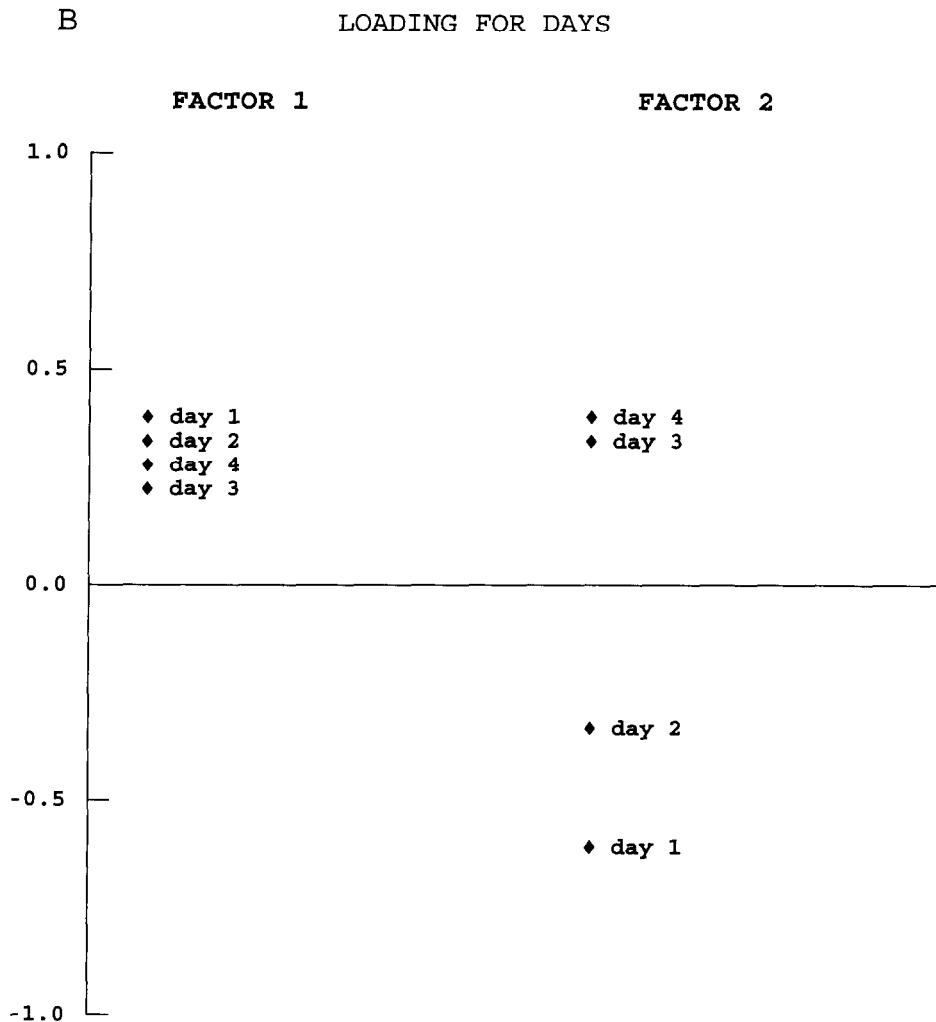


Fig. 4 (continued).

and of arbitrary factor rotations. The present method provides one solution which, over the test sessions, fits the data better than any other solution and provides an empirically grounded basis for selecting the best candidates for factors within a given domain.

The analyses, in the present study, of a data set derived from measures of open-field behaviors in rats during four different test sessions, indicate the existence of two general factors. One factor, 'emotional reactivity', which is characterized by defecation, urination, and avoidance of the center of the open-field, shows prominence on the first test session and then decreases in level. The second factor, 'exploratory activity' is characterized by activity in the center area of the open-field and gains in prominence as test sessions progress. These findings are in good agreement with previous studies of open-field rat behavior which used the more conventional two-way factor analytic approach. Royce

TABLE 2

Cross-products of normalized factors (i.e. cosines of the angles between the factors) for the two-, three-, and four-factor analyses with an orthogonality constraint on Mode B (days). Note that Mode A represents variables and Mode C subjects

	Factors			Factors		
	1	2		1	2	3
Mode A						
1	1.000	0.359	1	1.000	0.361	−0.246
2	0.359	1.000	2	0.361	1.000	0.167
			3	−0.246	0.167	1.000
Mode B						
1	1.000	0.000	1	1.000	0.000	0.000
2	0.000	1.000	2	0.000	1.000	0.000
			3	0.000	0.000	1.000
Mode C						
1	1.000	0.797	1	1.000	0.805	0.334
2	0.797	1.000	2	0.805	1.000	0.122
			3	0.334	0.122	1.000
	Factors					
	1	2		3	4	
Mode A						
1	1.000	0.397		−0.279	0.274	
2	0.397	1.000		0.157	0.162	
3	−0.279	0.157		1.000	−0.636	
4	0.274	0.162		−0.636	1.000	
Mode B						
1	1.000	0.000		0.000	0.000	
2	0.000	1.000		0.000	0.000	
3	0.000	0.000		1.000	0.000	
4	0.000	0.000		0.000	1.000	
Mode C						
1	1.000	0.793		0.403	0.508	
2	0.793	1.000		0.152	0.330	
3	0.403	0.152		1.000	0.185	
4	0.508	0.330		0.185	1.000	

(1977) summarized the findings from a number of multivariate examinations of open-field behavior in rats (Billingslea, 1942; Holland and Gupta, 1966; Singh, 1966; Whimbey and Denenberg, 1967a). In general, all of these studies obtained factors similar to emotional reactivity or autonomic balance (e.g. fear, timidity or elimination), as well as factors similar to exploratory activity or motor discharge (e.g. freezing, activity or withdrawal plus motor discharge). More recently, Markel et al. (1989) obtained a three factor solution of rat behavior in an open-field test. This study obtained 'exploration', 'fear', and 'shifted activity' factors in describing the observed behaviors.

The present results are also consistent with previous studies which have attempted to determine the validity of open-field measures (e.g. Ivinskis, 1970; Walsh and Cummins,

1976). These studies have suggested that defecation is a valid measure of emotional reactivity. It is also interesting to note that the negative thigmotaxis measure figured prominently in the obtained factors. On Day 1 the rats tended to stay in contact with the wall of the open-field apparatus, whereas, on Days 3 and 4 they were more likely to spend time in the center area of the open-field. Treit and Fundytus (1989) have suggested that thigmotaxis may be a 'prepared' fear reaction of rats and they provided pharmacological evidence for this. Anxiolytic drugs, such as diazepam and chlordiazepoxide, suppressed the behavior of staying near the wall of the open-field. Thigmotaxis (or occupancy of protected areas) also has been validated as a measure of emotionality in rats on the basis of genetic correlations (van der Staay et al., 1990).

The present findings, characterizing the temporal changes in prominence for the two proposed factors, also are consistent with previous studies which employed temporal analyses to examine open-field behavioral data (see Walsh and Cummins, 1976). Decreases in defecation over trials have been widely reported, and have been confirmed over as many as 60 trials (Bronstein, 1972). In addition, there have been reports of initial decreases, followed by increases, in rat open-field activity over repeated test sessions (e.g. Broadhurst and Eysenck, 1964), although this finding has been less consistent (Walsh and Cummins, 1976). Unfortunately, many studies have ignored temporal changes in open-field behaviors, and even when available, the data often have been collapsed over the test session dimension to avoid problems with data analysis. The present method provides an approach which incorporates these temporal changes as important features in the analysis.

Interpretation of open-field behavioral measures, as well as factors, has been rather problematic (see Walsh and Cummins, 1976, for a discussion of this point). Suarez and Gallup (1981) have argued that open-field testing entails both a predatory encounter (handling by the experimenter) and sudden social separation from conspecifics. Although the present study may have had a 'predatory' component influencing the initial testing (since handling was unavoidable under the present procedures), it should be noted that the animals were used to being handled, the experimenter was outside the curtains surrounding the open-field apparatus, and because of the position of the bright lights, not particularly visible to the subjects in the apparatus. Furthermore, the animals were individually housed for several months prior to the experiment, making 'sudden social separation' an unlikely influence in this case. Nevertheless, it would be useful to replicate the present procedure with a videotape method of data acquisition in an attempt to minimize such influences. It would be interesting, for example, to examine the effect of the experimenter's presence on the 'emotional reactivity' factor in rat open-field behavior.

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