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Author
Pisula, Wojciech

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SEQUENTIAL ANALYSIS OF RAT BEHAVIOR IN THE OPEN FIELD

Wojciech Pisula
University of Warsaw

ABSTRACT: Fifty four rats were tested in an open field. Both Frequency and Sequence of behavioral acts were analyzed. Distribution of the behavioral index frequencies appeared to be far from normal. Cluster analysis based on sequential data revealed that rats employ two main behavioral patterns in the open field. The results are discussed in terms of individual differences. The procedure used here represents an improved approach to analyzing open-field behavior.

INTRODUCTION

A frequently used procedure in the study of individual differences (ID) has been the observation of rats in the open-field test (Walsh & Cummins, 1976). In this widely used procedure, an animal is placed in a large arena and several behavioral measures are taken, including defecation, urination, rearing, ambulation, grooming, and pausing. It has been assumed that these behavioral traits are normally distributed and that their frequency or intensity are formal indicators of temperament (Strelau, 1987). Recent studies of individual differences conducted in our lab have focused on stimulus-seeking behavior (Matysiak, 1993; Pisula, Ostaszewski, Matysiak, 1992).

There is strong evidence that a univariate approach to the open-field test is not adequate (Tobach & Schneirla, 1962) and there are few attempts to improve upon these procedures (e.g. Tobach, 1976). Most studies suffer from a lack of sequential analyses. An analysis based solely on frequency does not permit us to identify typical action chains and a general strategy employed by animals in novel environments. It may be that the uncritical and general use of analytical techniques, such as ANOVA and R-Pearson coefficients, accounts for these

Address correspondence to Wojciech Pisula, Faculty of Psychology, University of Warsaw, Stawki 5/7, 00-183 Warsaw, Poland.

methodological problems.

It is our opinion that these statistical procedures limit a full understanding of the behaviors in question and interfere with the formulation of appropriate hypotheses about open-field behavior.

We have been lead to this view by the following:

- behavioral acts are not continuous, but are rather discrete; they either occur or not (intermediate states have been described as "intention movements" after McFarland, 1993)

- the same behavioral act may play a different role in different contexts (locomotion is observed during play, escape, or exploration)

- the meaning of the behavior depends on the sequence of individual behavioral acts, and not merely on their frequency.

The utility of the sequential analysis was demonstrated in recent study run by Makino, Kato and Maes (1991). We believe that a satisfactory analysis of individual differences should consider both qualitative and quantitative aspects of behavior. The purpose of the study reported here was to illustrate the value of both qualitative and quantitative analyses of open-field behavior.

METHODS

Subjects

Fifty four outbred male Wistar rats were tested. All were between 90-100 days of age.

Apparatus

The open field was similar to that described by Sherman et al. (1980). It measured 114 x 114 cm and had a floor made of white plastic tiles size 19 x 19 cm. In the center, 120 cm above the floor a 200 W bulb was suspended.

Procedure

We used procedures similar to those described by Sherman *et al.* (1980). An L-shaped barrier was placed in one corner of the field creating a cell in which the animal was placed. After 10 sec the barrier was removed and the animal was free to explore the field for 3 minutes.

Observations were recorded directly into a computer which allowed us to measure the frequency of behaviors as well as their sequence of occurrence. We recorded onsets of: *locomotion*, walking and running;

floor- and air-sniffing, movements of nose and whiskers; *rearing*, standing up with forelimbs in the air or against the wall; *grooming*, face washing, licking the body; *pausing*, a pause, often with mild head movements, between acts or interrupting an act; *defecation*, elimination of the fecal boli.

RESULTS AND DISCUSSION

Frequency

The first phase of the data analysis was an analysis of the distribution of the frequency of the dependent variables. This is shown in Table 1, in which it can be seen that most of the variables measured were not normally distributed. These is reflected in the skewness and kurtosis indexes presented in the table. There were two exceptions to this: floor-sniffing and locomotion in the first minute.

Table 1. Results of the analysis of the frequency distribution. Min, minute of experimental session; SD, Standard Deviation.

Variable	Min	Mean	SD	Kurtosis	Skewness
Floor Sniffing	1	9.61	3.72	0.46	0.05
	2	5.72	3.16	0.00	0.52
	3	3.56	3.03	4.34	1.62
Air Sniffing	1	1.54	1.41	3.29	1.43
	2	1.48	1.38	0.10	0.93
	3	1.00	1.06	-0.01	0.88
Grooming	1	0.22	0.50	4.51	2.25
	2	0.57	0.86	1.63	1.52
	3	0.19	0.52	16.37	3.66
Locomotion	1	4.43	3.05	-0.51	0.35
	2	2.22	3.12	1.62	1.57
	3	0.59	1.76	13.34	3.65
Rearing	1	4.54	2.71	-0.75	0.30
	2	1.85	1.99	0.41	1.01
	3	0.78	2.13	39.31	5.91
Pausing	1	0.44	0.74	1.96	1.63
	2	1.78	1.66	-1.04	0.45
	3	2.11	1.71	-0.23	0.55
Defecation	1	0.78	1.21	6.03	2.18
	2	0.91	0.90	0.11	0.84
	3	0.19	0.44	5.14	2.34

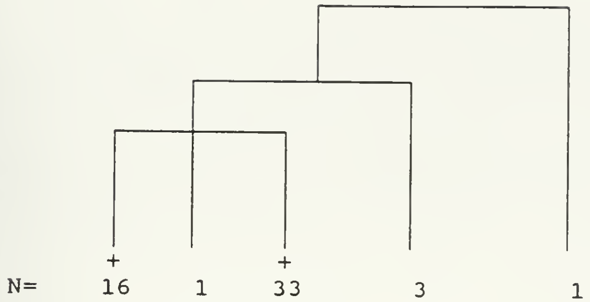

Figure 1. The results of the cluster analysis. Simplified dendogram presents revealed clusters of rats. Branches marked with "+" symbol illustrate clusters included in further analysis. Numbers illustrate cluster membership.

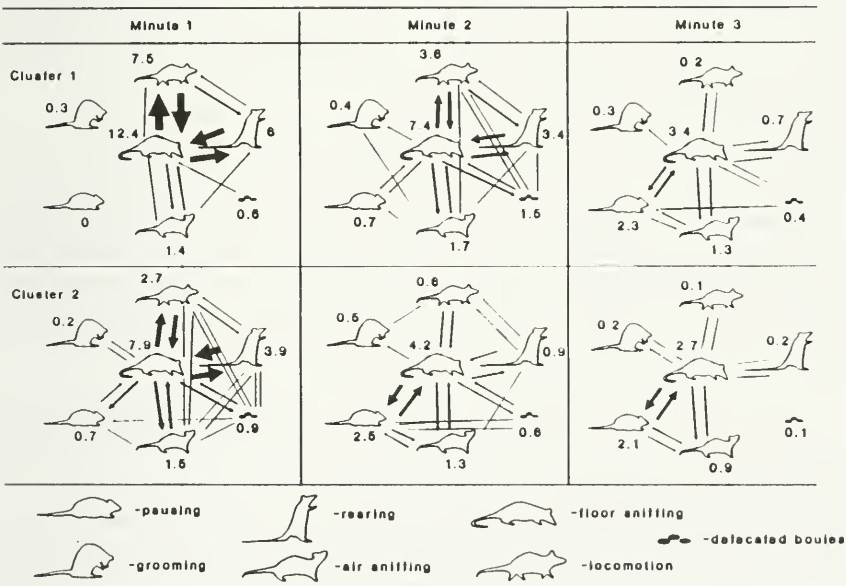

Figure 2. Results of the sequential analysis. Numbers near the images indicate mean frequency of given behavioural act. The thickness of the arrows indicates the proportion of the number of given transitions in one minute divided by the number of transitions in three minutes.

Sequence

All transitions from one behavior to another one were recorded, creating 42 variables for each 3-minute trial. An hierarchical cluster analysis (Between - Cluster Average Distance Method) was performed on these data and is summarized as a dendogram in Figure 1, which reveals two main clusters consisting of sixteen and thirty three rats. Behavioral patterns associated with each of these clusters are shown in Figure 2.

Cluster Characteristics

The animals tested in our study fell into two main behavioral clusters: Cluster 1 includes sniffing, locomotion, and rearing; in Cluster 2 behavioral acts were more equally distributed among all the behaviors recorded.

The patterns looked increasingly similar in minutes 2 and 3. The clearest IDS are seen during minute 1, reflecting perhaps diminishing stress over the course of the 3-minute trial. This is consistent with the view expressed by Strelau (1983), that temperament traits are most clearly seen in the most stressful situations.

Air/floor-sniffing, Locomotion and Rearing

Floor sniffing appeared to be the most common behavioral activity in all rats throughout the entire 3-minute session. This is in accordance with the data reported by Makino et al. (1991) for mice. It is worth noting that in our study sniffing appeared to be an intermediate state between all other behavioral forms measured. The transitions that did not include floor-sniffing were relatively rare.

With respect to locomotion, there were no incidences of running in this study. The rats walked around the open field, though some speed differences were apparent. Figure 2 shows that locomotion is strongly involved in the functional triangle along with sniffing and rearing. It is worth mentioning that locomotion and rearing are closely linked with sniffing and very poorly with each other. It would appear that this triad of behavioral activities, locomotion, sniffing, and rearing are closely related and are indicators of stimulus-seeking behavior motivated by a need for stimulation as discussed by Matysiak (1993).

Grooming

Grooming during exploration in rats is quite common and is discussed by Bindra and Spinner (1958). Grooming behavior was at one time classified as "displacement activities" (Marler & Hamilton III, 1966; McFarland, 1993), which are reported to occur in several situations: "a) physical thwarting of appetitive behavior, b) thwarting of consummatory behavior by removal of its object or goal and c) simultaneous activation of incompatible tendencies" (after McFarland, 1993, p. 414).

Our data show that grooming was linked with floor sniffing. It is most frequently seen during the second minute of the session, perhaps indicating some degree of motivational change.

Pausing

Pausing appeared to be linked with floor- and air-sniffing. There were no incidences of freezing observed in this study. Pausing tended to increase as the 3 min continued. This result contradicts the data of Makino et al. (1991) and may be the result of species (rats vs mice) and procedural differences (10 min session in Makino et. al study).

Defecation

Defecation has been thought to be a good indicator of emotionality or emotional reactivity (Broadhurst, 1957; Hall, 1934), although this generalization has been criticized (Tobach & Schneirla, 1962). Our data seem to support this criticism. As far as the sequence is concerned, defecation was almost randomly distributed over the 3 minutes of the trials. There was no clear association of defecation with other behavioral acts.

GENERAL DISCUSSION

Our data show that the distribution of the most commonly used behavioral indexes of rat behavior in the open field is far from being normal over the course of a three-minute trial in the apparatus. Some behaviors occur much more frequently in the early part of trials, others are distributed randomly over the entire course of the trial. This presents some difficulty in analyzing these behavioral characteristics as expressions of individual differences in temperament.

The general point we wish to make is that when analyzing behavior which is suggested to be pertinent to specific theoretical concepts (e.g. temperament) it is necessary to apply appropriate analytical methods. Most psychological concepts assume a high degree of complexity of processes described. Behavior characteristic of those concepts must themselves be somewhat complex. Statistical analyses of these behaviors must be of the sort capable of teasing out of these complexities. It is our suggestion that the typical ANOVA procedures are inappropriate for analyzing complex open field behaviors; they at best provide an incomplete assessment of the behavior involved.

The cluster and sequential analyses used in the present study are proposed as better procedures than have been typically employed in the past. They require far fewer prior assumptions and may help to reveal the most typical behavioral patterns. We believe that the components of the clusters of the sort identified here and the sequence of behavioral patterns we found is a function of the unique testing conditions in this study. It is our intention to investigate this suspicion in future studies.

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