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RAT BEHAVIOR IN THE EXPLORATION BOX - CLUSTER ANALYSIS

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ABSTRACT: The main objective of this study was to provide a description of the possible patterns of rat behaviour in the exploration box. Both age and sex of the animals were controlled. The measures taken assessed changes in the patterns of behavioural activity over time. Sex did not differentiate the rat's behaviour significantly. Two patterns of developmental changes were found. The results of cluster analysis with a sequential data sets lead to the conclusion that there are at least two levels of regulation of rat behaviour: activation and content organization. It was also shown that rat behaviour in the exploration box is not stereotyped but highly plastic. The results are discussed in terms of development, sex differences and individual differences.

The study of exploratory behaviour in the rat has implications for both the study of individual differences (Henderson, 1994; Pisula, Ostaszewski, Osiński, Trojan & Matysiak, 1995) and the development of psychological theory in general (Matysiak, 1992; Renner, 1990). There are several unsolved methodological issues in the study of exploratory behaviour. One is the selection of appropriate data analysis techniques. Another is the use of ecologically meaningful testing apparatus. The utility of multivariate analysis has been demonstrated in open-field behaviour (Pisula, 1994) and investigatory responses (Renner & Seltzer, 1994). It seems to be most useful in identifying behavioural strategies, particularly sequential analysis of behavioural transitions (Pisula, 1994; Renner & Seltzer, 1994). Thus it seems to indicate that the use of *a priori* indices are of little use. The use of multivariate techniques involving both quantitative and qualitative measures is more fruitful.

A second methodological issue concerns the appropriateness of the open field test. The complexity of behaviour manifested by the animal

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is, to some extent, a reflection of the complexity of the environment in which it functions. An open field test provides the animal with simplified conditions, which do not allow for initiating various forms of activity (Renner, 1990). Therefore, one may suppose, that the open-field behaviour is impoverished and does not reflect the complexity of behavioural repertoire characteristic for a given animal (Pisula, 1994). Thus, studies on individual differences manifested in spontaneous behaviour should involve test situations allowing animals to produce a greater diversity of behavioural acts. Such an apparatus was used in this study.

A third aim is to further investigate the behavioural correlates of the complex motivational mechanisms hypothesized to underlie exploratory behaviour (Matysiak, 1992). These correlates should reflect the complexity of the processes analyzed (Pisula, 1994).

The current study will also investigate sex and age differences with respect to exploratory behaviour. Both sex (Fitch & Dennenberg, 1995) and age differences (Bronstein, 1972; Oakley & Plotkin, 1975) have been often quoted. Renner, Bennet and White (1992) reported significant differences between rats of different age in various forms of exploration. However, their methodology was primarily aimed at investigatory and manipulatory responses. Therefore, it is important to confirm their findings using more general descriptors of exploratory behaviour.

METHOD

Subjects

Fifty five outbred Wistar rats were tested. The rats were grouped by sex with the following age breakdown; 5 subjects at 5 weeks old, 5 subjects at 10 weeks old, 5 subjects at 15 weeks old, 5 subjects at 20 weeks old. The male group had an additional 15 subjects at 25 weeks.

Apparatus

The box for exploratory behaviour measurement (Figure 1) was 42 x 32 x 40 cm. It was equipped with a see-saw, two ladders and two table tennis balls. All the things (except balls) were made of metal. Intensity of the white light (measured on the floor of the cage) was 50 lx.

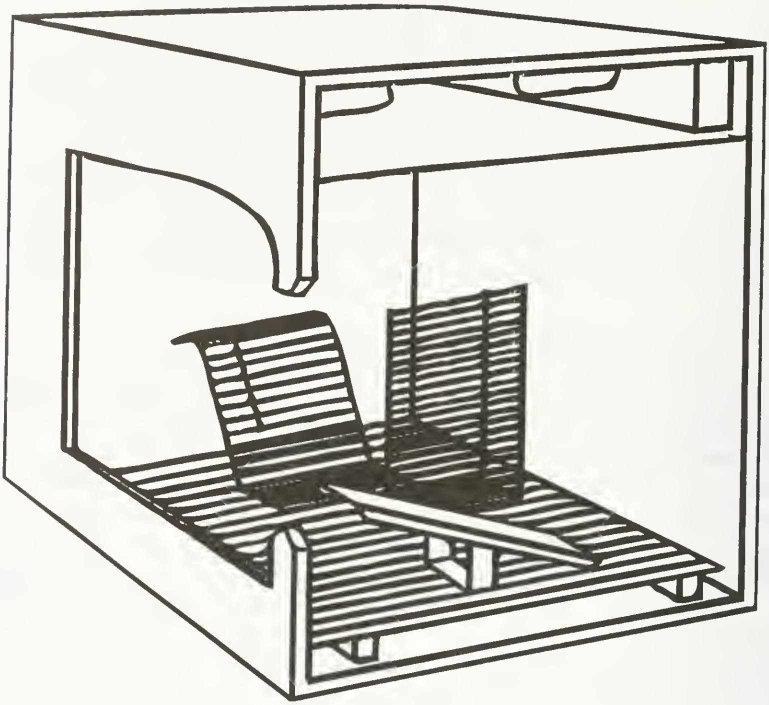

Figure 1. Exploration box used in this study.

Procedure

Each animal was placed individually in the exploration box for 60 minutes. All measurements were run between 6 to 8 pm under the light/darkness cycle 12/12h with turn on/off points at 8 am/pm. There was silence provided in the experimental room. Temperature was 20 ^{0}C. During the entire session the animal's behaviour was video recorded. The experimenter would leave the experimental room immediately after placing an animal in the exploration box.

Analysis of the video recordings

First, all behavioural acts were recorded in a two-dimensional table. The table reflected preceding (rows) and subsequent (columns) behaviours. Therefore, cell contents reflect the frequency of occurrence of a given sequence of behavioural acts. A trained observer analyzed the tapes frame-by-frame, classified behaviours and marked all of them in the appropriate cells.

There were three tables constructed for each animal reflecting three periods of measurement: the first-5 minutes duration, the second following-15 minutes duration, and the last-40 minutes in duration. The periods of measurement were selected on the basis of exploratory data. The onset times of twenty two behavioural forms were included in the tables as follows: walking (w); running (r); jumping (j); rearing (re); leaning against the wall (l) - rat stands on its hind-quarters and makes one- or two paw contact with the wall; leaning against the object (lo) - rat stands on its hind-quarters and makes one- or two paw contact with the object; grooming (g); floor- (fs) or object- (os) sniffing - rat puts nose close to or in contact with the floor or object; air sniffing (as) - subject moves nose upwards at a considerable distance from any object and is standing with at least three paws on the floor; gnawing (gn); body scratching (bs); lying down (ld); freezing (f); lying rolled up (lr); object touching (ot) - rat makes contact with objects with single or two paws; object manipulation (om) - rat makes contact with objects with single or two paws causing its movement; climbing the ladder (c); sitting (s); stretching (st); crawling (sc). Tables constructed for 22 behavioural forms had 484 cells.

For the purpose of further analysis (cluster analysis), each cell was treated as a new variable. Therefore, the behaviour of each animal was described by three sets (reflecting three periods of the measurement), each consisting of 484 behavioural indices.

RESULTS

Sex and age differences

Comparisons between sexes were limited to the age categories where sex ratios were the same. An initial analysis showed that subgroup variance was not homogeneous. Distributions of most indexes were far from being normal. Therefore nonparametric procedures such as: the U Mann-Whitney, Kruskal-Wallis ANOVA and CHI-square were applied. The exception was grooming, for which an exact Fisher test was performed. Comparisons were conducted for all periods of measurement independently.

During the initial 5 minutes of the session the females took sitting position more often than the males [$U = 127$, $N = 40$, $p < 0.05$]. Analysis of variance conducted for the time spent on grooming, in this period of testing, showed that the males groomed longer than the females [$F(1,30) = 6.0875$, $p < 0.05$]. During the next 15 minute period the females manifested gnawing more often than males [$U=114.5$, $N=40$, $p < 0.05$]. No sex differences were found during the last 40 minutes of the measurement.

Table 1 presents the age differences. Since the Kruskal-Wallis ANOVA was applied for this analysis, table shows ranked data for a given behaviour across time frames.

Table 1. Results of the analyses of age differences. Table includes only those behaviours that showed significant differences across time frames. The frequency of each behavioral act was ranked in ascending order. Cells show mean ranks in age subgroups. Expected ranking in each cell=20.5. Part A of the table shows the results of the first (5 min long) part of the session. Part B represents the next 15 minutes of the session and part C the last 40 minutes of the session. See "Procedure" for behaviour description.

Time	Behaviour	Mean Rank of Behaviour Frequency				χ^2	P
		5-weeks	10-weeks	15-weeks	20-weeks		
A	W	34.80	24.6	14.05	8.50	29.92	0.01
	L	23.45	26.45	19.40	12.7	7.84	0.05
	G	33.20	18.00	15.40	15.40	17.83	0.01
	FS	17.10	27.70	23.50	13.70	8.72	0.05
	W	30.70	21.40	13.19	8.50	23.19	0.01
B	LO	22.40	23.70	25.90	10.00	11.25	0.01
	FS	17.85	26.55	26.65	10.95	12.65	0.01
	AS	15.25	21.80	28.45	16.50	7.96	0.05
	GN	25.55	22.33	20.55	11.80	8.15	0.05
C	W	20.10	25.00	24.05	9.83	10.67	0.05
	LO	11.65	26.35	25.35	18.65	10.33	0.05
	FS	8.00	28.30	27.85	17.85	20.37	0.01
	OS	12.05	25.00	24.11	19.25	8.05	0.05
	AS	8.85	29.15	26.70	17.30	19.02	0.01
	GN	18.40	28.75	20.95	13.90	8.62	0.05
	S	12.00	18.35	28.55	23.10	10.91	0.05

Cluster analysis

Hierarchical cluster analysis (Ward's method) was conducted to identify the main behavioural patterns shown by the rats. The analysis was run on the sequential data (484 cells of the transition table). There were three analyses conducted for three periods of measurements (1 per period). The results are summarized in Figure 2.

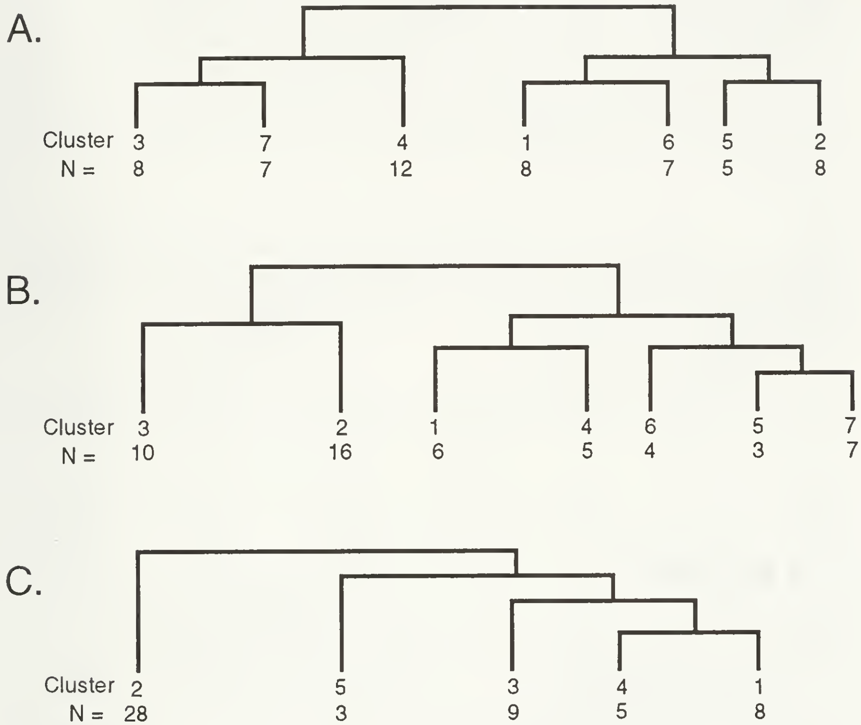

Figure 2. Inversed dendrograms illustrating results of cluster analysis of the experimental session, broken into three time periods: A., The initial 5 mins (0-5mins); B., the following 15 mins (5-20mins); and C., the last 40 mins (20-60 mins). At the ends of the branches, reflecting clustered groups of rats, are shown the cluster numbers and the cluster sizes (N).

The relationship between sex and cluster membership was analyzed using CHI-square statistics, assuming the ratio 20 females / 35 males. Table 2 shows the results of this analysis.

Age and cluster membership relationship were analyzed with Kruskal-Wallis ANOVA. The results are shown in Table 3. Figure 3 shows behavioural patterns presented by the extracted.

Each animal could belong to multiple clusters, because a separate cluster analysis was conducted for each time period. The cluster membership of each subject is provided below. Animals are described with three digit codes. The first digit stands for cluster number extracted during the initial 5-min measurement, the second digit stands for following 15-min of the session, and the third one for third interval cluster. An "x" was used to indicate clusters with only one subject. These clusters are not shown in Figure 2. Cluster membership obtained is as follows: 11x, 111, 112, 123, 123, 124, 132, 141, 222, 222, 223, 242, 264, 272, 272, 272, 3x2, 311, 322, 322, 322, 322, 323, 332, 4x5, 422, 422, 423, 423, 425, 432, 432, 432, 432, 433, 454, 511, 54x, 562, 564, 564, 6x1, 6x3, 611, 643, 645, 651, 651, 732, 732, 732, 772, 772, 772, 772.

Table 2. Sex ratio in each cluster. Obtained Ratio, female/male proportion obtained in the clusters; Expected Ratio, expected ratio for a given cluster size.; * $p < 0.05$.

Cluster	Obtained Ratio			Expected Ratio		
	5-min	15-min	40-min	5-min	15-min	40-min
I	3 / 5	3 / 3	2 / 6	2.9 / 5.1	2.2 / 3.8	2.9 / 5.1
II	3 / 7	5 / 11	8 / 20	3.6 / 6.4	5.8 / 10.2	10.1 / 17.9
III	1 / 7	0 / 10	2 / 7	2.9 / 5.1	3.6 / 6.4	3.2 / 5.8
IV	4 / 8	2 / 3	5 / 0	4.3 / 7.7	1.8 / 3.2	1.8 / 3.2
V	5 / 0	2 / 1	1 / 2	1.8 / 3.2	1.1 / 1.9	1.1 / 1.9
VI	1 / 6	4 / 0	-	2.5 / 4.5	1.4 / 2.6	
VII	3 / 4	3 / 4	-	2.5 / 4.5	2.5 / 4.5	
χ^2 for difference between Expected and Obtained Ratios				12.37	14.55*	10.92*

Table 3. Mean age rank in extracted clusters. Age was ranked in a ascending order. Expected mean rank in each cell is 28. *p < 0.01.

Cluster	5-min	15-min	40-min
I	34.8	22.1	20.2
II	15.5	34.0	28.7
III	42.1	39.9	35.0
IV	34.9	25.2	17.2
V	15.5	15.0	21.8
VI	19.8	10.3	-
VII	23.7	5.5	-
χ^2	21.0*	34.9*	6.9

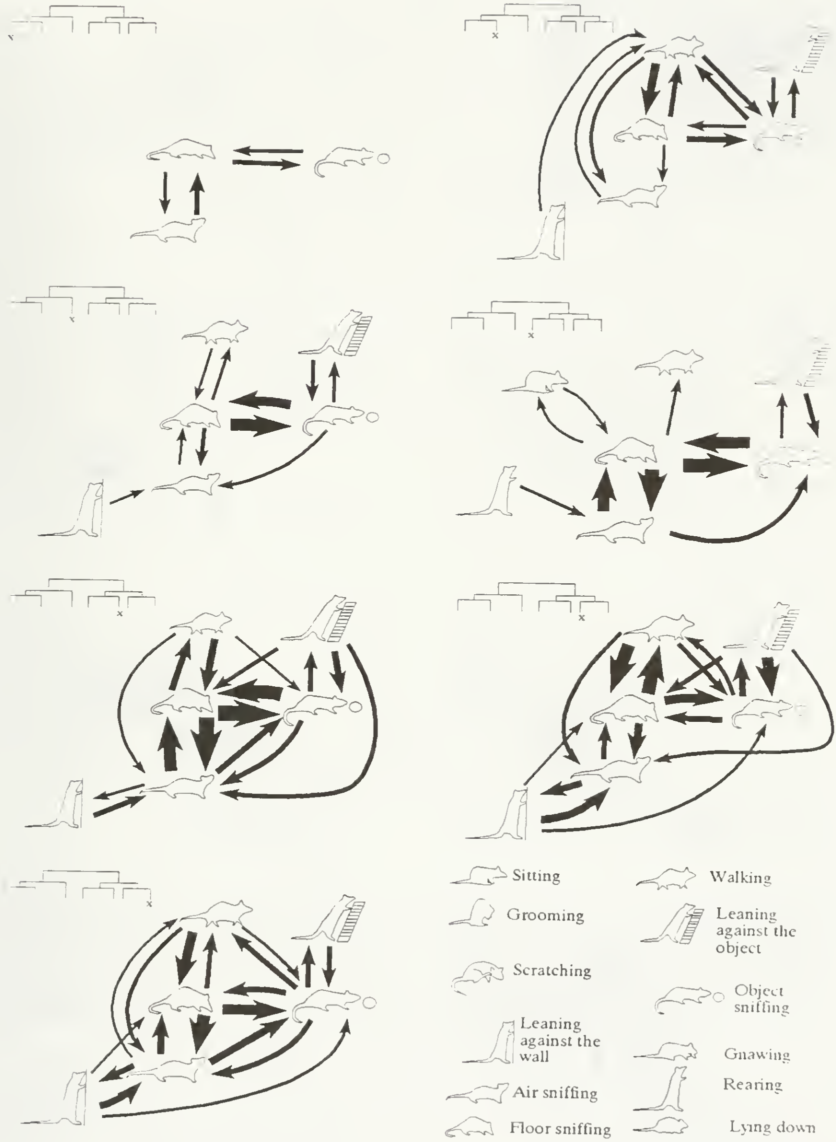

Figure 3a. Behavioral patterns during the first 5 minutes of the experimental session presented by rats of different clusters. The thickness of the arrows illustrates the frequency of the transition. Small dendrograms provided near the images inform about the cluster position within the whole sample.

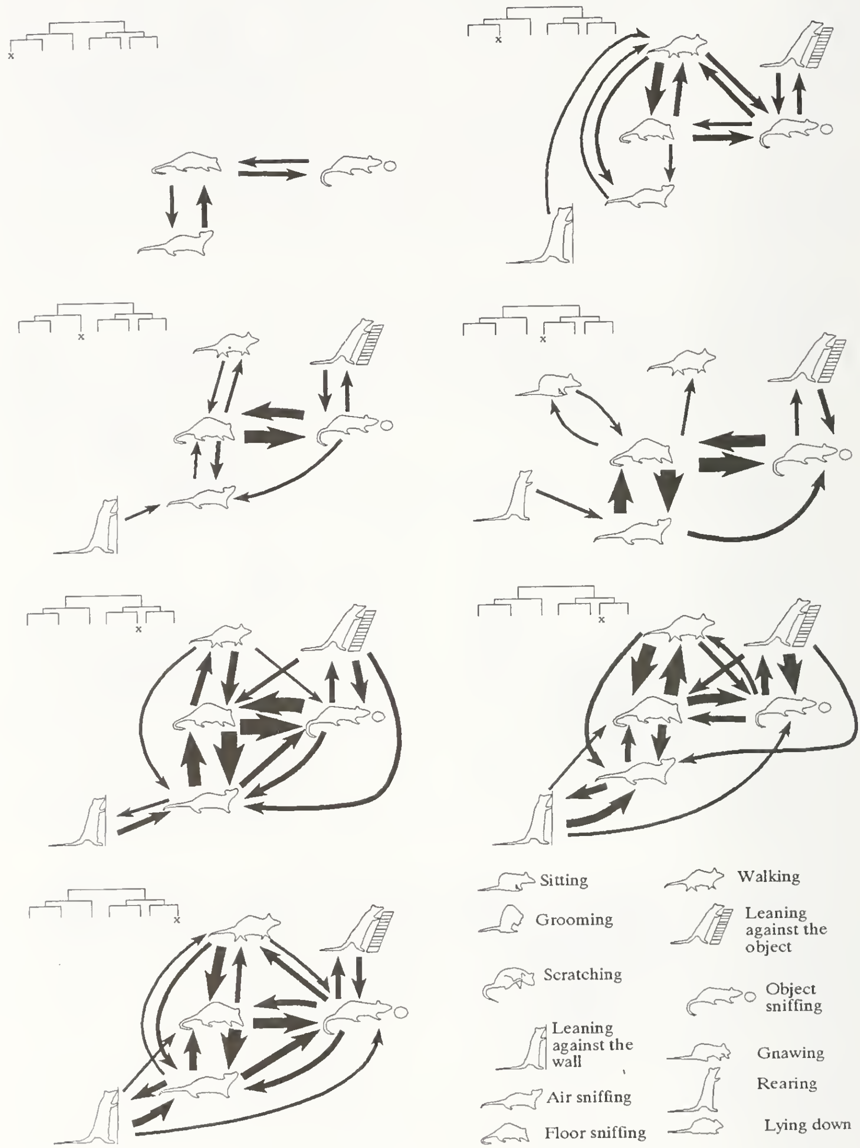

Figure 3b. Behavioral patterns during the 5-20 min period of the experimental session presented by rats of different clusters. The thickness of the arrows illustrates the frequency of the transition. Small dendrograms provided near the images inform about the cluster position within the whole sample.

Figure 3c. Behavioral patterns during the last 40 minutes of the experimental session presented by rats of different clusters. The thickness of the arrows illustrates the frequency of the transition. Small dendrograms provided near the images inform about the cluster position within the whole sample.

DISCUSSION

Sex and age differences

Gray (1971) found that female rats were more active and less fearful than the males. These results have been cited widely though they have lacked clear empirical confirmation. In a previous study conducted in our laboratory (Ostaszewski & Pisula, 1994) we analyzed the individual differences in open field behaviour. In three strains (DA/Han, August, Long-Evans) females were found to be more active in the open field than males. In the case of WAG rats this relationship was reversed. Gray and Lallje (1974) also found similar effects. An interesting sex difference was found in grooming behaviour. Grooming was classified as "displacement activity" and is interpreted as an expression of internal conflict (Bindra & Spinner, 1958; McFarland, 1993). Thus, a longer time spent on grooming in males may reflect stronger internal conflict. It is still necessary to determine what motivational components, or what kind of mechanisms underlie this effect. It is probably a multilevel phenomenon. On the physiological level, female gonadal hormones may play a role (Denti & Negroni, 1975; Stewart & Cygan, 1980). On the other hand, male rats spend more time on competition and hierarchical behaviour than females (Colhoun, 1963). Experience of pain and losses may increase the general level of anxiety. Although some sex differences were found in this study, they do not appear to be as meaningful as developmental factors. This effect confirms the results obtained by Renner, Bennett and White (1992).

There were two main patterns of developmental change found in this study. The first one is a continuous decline of a given form of activity as the rats get older. This is most clearly seen in: walking, grooming and also gnawing. Conversely, activities such as: sniffing, leaning against the wall, leaning against the object, present quite different dynamics. In these cases, we can see an increase and subsequently decrease of the level of activity.

The developmental effects found in walking and grooming correspond with earlier findings. Bronstein (1972) found that open field activity in rats declined, beginning from the fifth week of life. Bronstein considered a square entered, when the head and at least one forepaw moved across the line. This, perhaps, is an insufficient measure. It leads to record ambivalent behaviour (moving head and forepaws forward and backward) as a locomotion. As a result of this procedure behavioural activities controlled by different motivational mechanisms may be combined. Nevertheless, a decrease of motor activity in rats after the fifth week of life is well confirmed. A developing rat presents the peak activity at the age of 20-30 days. This period is connected with maturation of the mesencephalon (Oakley & Plotkin, 1975) and intensive play (Colhoun, 1963).

A quite different effect was found with various forms of sniffing. The term "sniffing" is a simplification in this study. We included in this category both vibrissae and nose working. Sniffing is directly involved in information seeking. The highest level of that activity was shown by 10- and 15-week-old rats. This result corresponds well with Renner's et al. (1992) work, which reported increase of sniffing behaviour in rats up to the age of 90 days. Hence, the subsequent decrease in this activity (after 15th week of life), found in this study is worth noting. A similar pattern was shown in leaning against the wall. Again, Renner at al. (1992) reported an increase in rats of age of 60 days, and subsequent decrease in older rats, which is in accordance with the results of this study, in which 10-week-old rats showed a peak in this activity. This pattern of developmental dynamics may reflect a great change in the rats' lives at this age. At the age of 70 days rats initiate sexual activity. At the age of 90-100 they are fully matured (Sokolov & Karasjova, 1990). This is a very stressful period in a rat's life. Males begin their struggle for a position in the colony. This process results in increased dispersal (Colhoun, 1963). Therefore, survival depends on the ability to recognize the threats coming from the environment. The observed decrease of sniffing in 20-week-old rats may be interpreted as a function of stabilization in the animal's development and adjustment to the environmental demands. This interpretation has to be verified experimentally.

The measures of contacts with objects indicated differential developmental trajectories. For example gnawing was found to decrease with age. Conversely, leaning, sniffing and leaning against the wall increased with age. Current data does not lead itself to an interpretation of those patterns. Further study is required in this regard.

Sex and cluster membership

The role of sex differences in cluster formation was not clear. There was no relationship between the sex and cluster membership during the initial five minutes of the session. The sex factor became apparent in the subsequent 15- and 40-minute time periods. It may be that in novel (often threatening) situations the mechanisms governing behaviour, common to all individuals, are decisively predominant. The role of the factors linked with sex increases as the organism adjusts to the environment.

Age and cluster membership

A strong relationship between cluster formation and the age of the animals was found in this study. This effect corresponds well with the previous findings (significant age differences described above). Interestingly, the relationship between age and cluster membership disappeared in the 40-minute time period (see Table 3). One may suppose that developmental factors influence individual differences in behaviour in novel situations. Age dependent differences remained also in the third (40 min long) phase of measurement, but the relationship between age and cluster formations declined. This means that in a further phase of the measurement, besides the remaining behavioural quantitative differences among the animals of different age categories, the role of developmental factors in defining behavioural qualitative differences, which are also responsible for cluster formation, disappears.

Characteristic of cluster formation

Cluster formation indicated the presence of two main rat behaviour strategies. This was apparent in all the three analyses. Comparison of the left and right main (top) branches of the reversed dendrograms leads to the conclusion that the left ones reflect a lower level of overall activity, whereas the right ones illustrate a higher level of activity. Therefore, overall activity seems to be the main factor differentiating the animal's behaviour. This result corresponds with a previous, open-field finding (Pisula, 1994). However, the behaviour shown by animals in the exploration box in this study was far more complex than that in the open field. This effect supports our opinion that an open field test, in its widely used version, can impoverish the animal's behaviour. Thus, these data seem to support the idea that environmental complexity, understood as the potential complexity of relationships (contingencies) between the behavioural acts and changes in the environment, seems to be an important factor that influences behaviour (Kuo, 1967).

There seems to be no immediate relationship between the level of overall activity and qualitative pattern of behaviour in this study. Both high- and low- active clusters of rats presented complex behavioural patterns as well as extremely simple ones. These patterns differed in their content. On the other hand, there were patterns of similar content shown by the animals at a quite different level of overall activity. The regularities of the clustering process found can be summarized as the following three observations. Cluster formation involved both quantitative and qualitative differences in the animals' behaviour. The primary factor in cluster formation is overall level of motor activity. At a relatively equal level of motor activity, the secondary (qualitative) differences influence the process of cluster formation.

The characteristics of cluster formation listed above allow me to formulate a view on behavioural processes governing rat behaviour in the exploration box. There are at least two levels of behaviour control processes. The first one is associated with arousal. There is general consent as to the neurophysiological basis of arousal: it is the function of the Reticular Activation System (RAS) of mesencephalon (Eysenck, 1967; Routtenberg, 1968). The classic work by Glickman, Sroges and Hunt (1964) supports this view. These authors tested the effects of lesions of different levels of central nervous system. The most evident changes in exploratory behaviour were obtained after mesencephalon lesions. On the other hand, Oakley and Plotkin (1975) showed that the highest level of activity in rats is strongly correlated with mesencephalon maturation.

The second level of behaviour regulation involves content organization. At this level, the direction of behaviour is determined. While in the first case the main point is to define an activational aspect of behaviour shown in every form of activity, in the case of the content analysis, it is important to orientate the behaviour with regard to the external environmental stimulus, to select the form of behaviour, and to allocate it in time. This level of behaviour regulation seems to be based on the limbic system (Routtenberg, 1968) and frontal cortex (Kolb, 1984). Therefore it is a higher level of regulation, linked with phylogenetically younger parts of CNS.

Renner and Seltzer (1994) discussed their finding in terms of a possible stereotyped nature of exploratory behaviour. They presented the data supporting the view that, at least in rats, exploratory behaviour is definitely not stereotyped. The results of cluster analysis obtained in this study also support this view. Though rats formed certain types of behavioural activity, reflected by extracted clusters, it is hard to say that they were stereotyped. The analysis of the flow of rats between clusters extracted in different periods of measurement leads to the conclusion that they can utilize different patterns of behaviour at different times and at different levels of general activity. Thus, the presence of an inherited mechanism governing the structure of the behaviour in the exploration box seems to be unlikely.

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REFERENCES

Bindra, D. & Spinner, N. (1958). Response to a different degrees to novelty: The incidence of various activities. *Journal of Experimental analysis of Behavior, 1,* 341-350.

Bronstein, P.M. (1972). Open-field behavior of the rat as a function of age. *Journal of Comparative and Physiological Psychology, 80,* 335-341.

Brown, R.E. & McFarland, D.J. (1979). Interaction of hunger and sexual motivation in the male rat: A time-sharing approach.*Animal Behaviour, 27,* 887-896.

Calhoun, J.B. (1963). *The ecology and sociology of the norway rat.* Bethesda, Maryland: U.S. Department of health, education, and welfare.

Denti, A. & Negroni, J. (1975). Activity and learning in neonatally hormone treated rats. *Acta Physiologica Latinoamerica 25,* 99-106.

Eysenck, H.J. (1967). *The biological bases of personality.* Springfield: C.C. Thomas.

Fitch, R.H. & Denenberg, V.H. (1995). A role of ovarian hormones in sexual differentiation of the brain (56 paragraphs, 1159 lines, 93 references). *Psycoloquy.95.6.05.sex-brain.1.fitch.* Available E-mail: psyc@pucc Message: Get psych 95-6-05

Glickman, S.E. Sroges, R.W. & Hunt, J. (1964). Brain lesions and locomotor exploration in the albino rats. *Journal of Comparative and Physiological Psychology, 58,* 93-100.

Gray, J.A. & Lalljee, B. (1974). Sex differences in emotional behavior in the rat: correlation between open-field defecation and active avoidance. *Animal Behaviour,* 22, 856-861.

Gray, J.A. (1971). Sex differences in emotional behavior in mammals including man: endocrine bases.*Acta Psychologica, 35*, 29-46.

Henderson, B. (1994). Individual differences in experience-eroducing tendencies. In: H. Keller, K. Schneider & B. Henderson (Eds) *Curiosity and exploration* (pp. 213-225). Berlin, Heidelberg, New York: Springer Verlag.

Kolb, B. (1984). Functions on the frontal cortex of the rat: a comparative review. *Brain Research Reviews, 8,* 65-98.

Kuo, Zing-yang (1967). *The dynamics of behavior development: An epigenetic view.* New York: Random House.

Matysiak, J. (1992). Theory of need for stimulation. *Polish Psychological Bulletin, 23,* 363-370.

McFarland, D. (1993). *Animal behaviour: psychobiology, ethology and evolution.* London: Longman.

Oakley, D.A. & Plotkin, H.C. (1975). Ontogeny of spontaneous locomotor activity in rabbit, rat and ginea pig. *Journal of Comparative and Physiological Psychology, 89,* 267-273.

Ostaszewski, P. M. & Pisula, W. (1994). Sex and strain differences in anxiety, emotional reactivity, exploration, and need for sensory stimulation in rats. *Polish Psychological Bulletin, 25,* 137-146.

Pisula, W. (1994). Sequential analysis of rat behavior in the open field. *International Journal of Comparative Psychology, 7,* 194-201.

Pisula, W., Ostaszewski, P.M., Osiński, J.T. Trojan, M. & Matysiak, J. (1995). Exploratory behavior in comparative psychology of individual differences. *Polish Psychological Bulletin, 26,* 239-250.

Renner, M.J. (1990). Neglected aspects of exploratory and investigatory behavior. *Psychobiology, 18,* 16-22.

Renner, M.J., Bennet, A.J. & White, J.C. (1992). Age and sex factors influencing spontaneous exploration and object investigation by preadults rats (*Rattus Norvegicus*). *Journal of Comparative Psychology, 106,* 217-227.

Renner, M.J. & Seltzer, C.P. (1991). Molar charateristics of exploratory and investigatory behavior in the rat (*Rattus norvegicus). Journal of Comparative Psychology, 105,* 326-329.

Renner, M.J. & Seltzer, C.P. (1994). Sequential structure in behavioral components of object investigation by Long-Evans rats (*Rattus Norvegicus*). *Journal of Comparative Psychology, 108,* 335-343.

Routtenberg, A, (1968). The two-arousal hypothesis: Reticular formation and limbic system. *Psychological Review, 75,* 51-80.

Sokolov, V.E. & Karasjova, E.V. (1990). *Norway Rat: systematics, ecology, regulation of its population.* Moscow: Nauka Publishers.

Stewart, J. & Cygan, D. (1980) Ovarian hormones act early in development to feminize open field behavior in the rat. *Hormones and Behavior 14,* 20-32.