Microstructural Assessment of Rodent Behavior in the Hole-Board Experimental Assay

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ABSTRACT

The aim of the present methodological article was to assess whether a microstructure of hole-exploratory activity can be demonstrated in rats tested in the hole-board, an experimental assay widely used to study anxiety-related behaviors in rodents. Six never properly described hole-exploratory behavioral components were identified: active-dip, static-dip, rapid-dip, circular-sniff, point-sniff and central-sniff. Results, assessed by means of descriptive and multivariate approaches, reveal a complex microstructural organization of these behavioral elements. The hypothesis of an important role of the relationships between point-sniff and head dipping activities, in anxiety-related hole-exploratory behavior, is proposed. It is suggested that the microstructural analysis can evidence even subtle behavioral changes induced by manipulations of animal anxiety condition.

Author Keywords

Multivariate analysis, stochastic analysis, hole-board, rat.

ACM Classification Keywords

I.2..1 Applications and expert systems: Medicine and science, G.3 Probability and statistics: multivariate statistics, H.5.2 User interfaces: theory and methods

INTRODUCTION

The hole-board (HB) is an exploration-based assay widely used to assess various features of anxiety-related behaviors in rodents. HB generally consists of a rectangular or square arena provided with a variable number of holes in the ground. The behavioral component more frequently investigated in HB is head-dip, that is, insertion of the head into a hole. However, conflicting evidences surround this behavioral component. For instance, increases [18], decreases [14], or no modifications [16] of head-dip frequencies have been described following administration of anti-anxiety drugs. Thus, whether quantitative evaluation of head-dip alone may be considered suitable anxiety level indicator represents matter of discussion. It is our contention that contrasting findings concerning this behavioral component have been produced by the unfortunate association of two factors: first, since HB introduction almost 50 years ago [1], inter-relations among behavioral components in this experimental assay have been scantly investigated; second, as a consequence and even more important, only quantitative evaluations of head-dip have been produced for a long time. Multivariate analyses (MVA) provide excellent tools to overcome such constraints because they make available useful and interesting information on the relationships among behavioral elements [3, 4, 5, 10, 11, 15, 17]. Concerning studies on anxiety and depression, various articles have provided, by means of MVA, reliable information on rodent's behavior in the elevated-plus maze [9], in the forced swimming test [12], in the sucrose preference test and in the open field [2]. As to hole-board assay, by means of MVA, we have demonstrated that edge-sniff, a specific sniffing activity of holes edge, has complex relationships with head-dip and with several components of the behavioral structure [6, 7, 8]. In brief, we hypothesized that the patterning between these two behavioral components could rely on rodent's motivational drives which, in turn, are likely influenced by anxiety level [6, 7, 8]. Thus, given the prospective functional meaning of head-dip/edge-sniff patterning, the study of hole exploration activities, at a microstructural level, might contribute to the knowledge of motivational drives underlying anxiety-related behaviors in

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HB. On this subject, remarkable information has been provided, in the past, by analyzing the behavioral microstructure of different rodent's activities, such as grooming [11] or feeding [20]. The aim of the present research is to describe, from a methodological point of view, an alternative approach to the study of rodent behavior in HB, by focusing the analysis on hole-exploration activity in its microstructural complexity.

METHODS

Experimental Apparatus

HB apparatus consisted of an enclosed 50 × 50 cm arena made of white opaque Plexiglas with a raised floor (5 cm above a white opaque Plexiglas sub-floor) containing four equidistant holes (4 cm in diameter). Each hole centre was 10 cm from the two nearest walls so that holes were equidistant from adjacent corners [6, 7, 8].

Subjects and Housing

15 male, specific pathogen free, Wistar rats (Morini, Italy), 55-60 days old, weighing 220-250 g, were used. Each subject was individually housed in a room maintained at 23 ± 1°C, under a normal 12 h light/dark cycle (light on: 7:00 a.m. – light off: 7:00 p.m.). Food (standard laboratory diet - Mucedola, Italy) and water were available "ad libitum".

Procedure

Rats were transported from housing room to testing room inside their home-cages to minimize transfer effect. To avoid possible visual and/or olfactive influences, animals were allowed to acclimate for 30 minutes far from observational apparatus. Environmental temperature was maintained equal to temperature in the housing room. Each subject, experimentally naïve at test beginning, was placed in the arena centre and allowed to freely explore for 10 min. After each observation, hole-board apparatus was cleaned with ethylic alcohol (70%) to remove scent cues left from the preceding subject. Experiments were recorded through a digital videocamera and video files stored in a personal computer for following analyses.

Ethical Statement

Experiment was approved by a local veterinary committee and conducted in accordance with the European Communities Council Directive 86/609/EEC concerning the protection of animals used for experimental scientific purposes.

Ethogram and Behavioral Coding

The first step in a behavioral study is generally represented by the construction of a suitable ethogram, that is, a formal description of the behavioral components taken into consideration. The ethogram here used is focused on rodent's activities of hole-exploration. Since behavioral coding is an extremely work-demanding aspect, utilization of a suitable software coder is very important. We analyzed digital video files by means of the software coder The Observer (Noldus Information Technology, The Netherlands) [19].

Descriptive Analyses

To discern frequent behavioral components from the infrequent ones per cent distributions have been calculated and presented by means of a pie-chart (Figure 1). Moreover, latencies of each behavioral element first appearance have been evaluated and graphically presented by means of a histogram (Figure 2).

Multivariate Analyses

Different multivariate approaches are available to assess animal behavior [5, 8, 10, 11, 15, 17]. In present research we used a MVA based on transition matrices elaborations. First of all, transitions from a behavioral element to another one were reported in a transition matrix (TM) by means of a relevant option available in the software coder. Then, TMs of each subject were summed obtaining a total TM. Within total TM, two important conditions should be respected: a) number of empty cells should be not more than 20% [4, 5, 6]; b) minimum number of transitions should be, at least, 5 times the number of components in the ethogram [5, 17]. Total TM represents the starting point for several analyses based on matrix elaboration such as stochastic analysis, cluster analysis or adjusted residuals analysis [5]. In this brief article, we present only the stochastic one. Multivariate stochastic analysis requires that a total TM is transformed into a table presenting probabilities of transitions among patterns (i.e. a probability matrix) where: a) transition probability from a pattern to all others is 1; b) each row must sum to 1, and c) each transition must be between 0 and 1 [4, 5, 6]. In present study individual TMs, along with resulting total TM, were processed by means of the specific software for matrix manipulation and analysis Matman (Noldus Information Technology, The Netherlands) [13].

MVA Graphical Representations

Basically, a transition matrix is a table filled with hundreds of numbers. In this form a transition matrix is quite difficult to interpret even for an experienced reader. For this reason it is important to express a transition matrix in an intuitive graphical fashion. In present article, the stochastic matrix has been graphically expressed by means a path diagram in which different transition probabilities were represented by connecting arrows of different thickness. By means of this approach both directions and transition probabilities can be simultaneously illustrated.

RESULTS

Ethogram of the Hole-Exploration Process

The ethogram encompasses six behavioral components: Point-Sniff (PoS): the rat sniffs a single point of the hole-edge; Central-Sniff (CtS): the rat sniffs the hole center without inserting the head inside; Circular-Sniff (CiS): the rat sniffs the hole-edge in a continuous circular fashion;

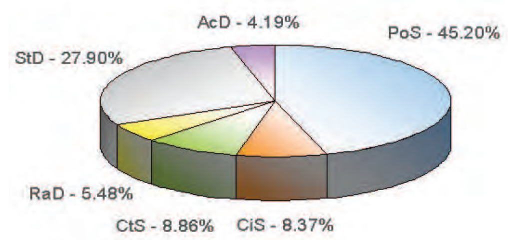

Figure 1. Per cent distribution of rat's hole exploratory components. Abbreviations in the text.

Static-Dip (StD): the rat puts and maintains its head into the hole, the body is immobile; Rapid-Dip (RaD): the rat rapidly puts into and remove its head from the hole; Active-Dip (AcD): the rat puts its head into the hole; body movements are produced. Behavioral group "others" enfolds all remaining components not related with hole-exploration [6, 7, 8].

Descriptive Analyses

Figure 1 shows that all sniffing activities, that is, point-sniff, circular-sniff and central-sniff, represent together the largest extent of hole-exploratory patterns. Mean latencies (Figure 2), evaluated on the basis of each behavioral component first appearance, demonstrate that point-sniff and static-dip are the first components of hole-exploratory behavior to appear, both within the first minute of observation. Rapid-dip, active-dip and circular-sniff are present after the first minute and within the third. Central-sniff is the last pattern to occur.

Multivariate Stochastic Analysis

Probabilities of transitions, illustrated by means of a path diagram (Figure 3), show that all dipping behaviors (i.e. rapid-dip, static-dip and active-dip) have high chances of transition exclusively toward point-sniff. This last, in turn, represents the only pattern linking the remaining components of the behavior (i.e. "others") with a high probability. All remaining transitions occur with medium to low probabilities.

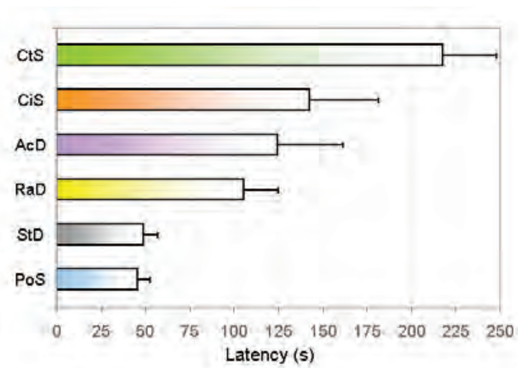

Figure 2. Latencies ± SE (s) of each behavioral component first appearance. Abbreviations in the text.

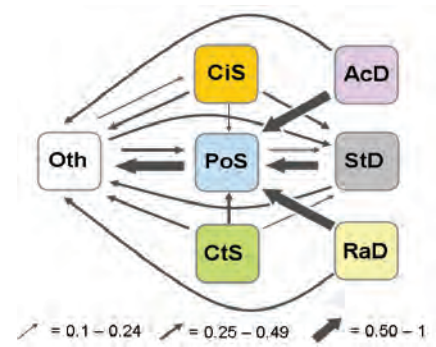

Figure 3. Path diagram illustrating three different transition probabilities. Abbreviations in the text.

DISCUSSION

This is the first research assessing, by means of descriptive and multivariate analyses, the behavioral process of hole-exploration at a microstructural level. Six new behavioral components, never properly described, have been introduced and two main behavioral clusters of hole exploration activities in HB were identified: the sniffing one and the inside-hole exploration one. The former consisted of point-sniff, circular-sniff and central-sniff; the latter encompassed rapid-dip, static-dip and active-dip.

The pie-chart (Figure 1) shows a prevalence of all sniffing activities in comparison with the inside-hole exploratory ones. Noticeably, point-sniff and static-dip represent roughly the 70% of the whole behavioral repertoire of hole-exploration. Latencies (Figure 2) demonstrate that point-sniff and static-dip are the first to appear, both within the first minute of observation. All the remaining components show higher latencies. Path diagram reveals that all head-dipping activities have high transitional probabilities exclusively toward point-sniff (Figure 3) that, in turn, is the sole behavioral element of hole-exploration process showing high transition probabilities toward remaining components (i.e. 'others'). Taken together, such results suggest an important role of point-sniff, static-dip and of their patternings, both in terms of quantitative aspects and probabilistic arrangement of transitions as well. However, per cent distributions, latencies and, more in general, all quantitative assessments of behavior are able to emphasize only specific quantitative aspects, representing each component, although carefully quantified, only a disjointed element. For instance, albeit pie chart (Figure 1) clearly demonstrates that point-sniffing is characterized by a high per cent value and by the lowest latency (Figure 2), relationships among point-sniff and the whole behavioral structure are missing. Hence, the possibility to characterize each behavioral component through even thousands of numbers does not imply the possibility to use those numbers to figure out what the behavior is in its wholeness. As already showed in other experimental models of anxiety and depression [2, 9, 12], this is the pivotal aspect that distinguishes 'conventional' quantitative approaches from multivariate ones: MVA offers useful tools to avoid reductive interpretation of isolated patterns, disjointed from the whole behavioral architecture, thus revealing behavioral dynamics otherwise undetectable by means of quantitative analyses alone.

CONCLUSION

Main outcome of the present research is that rat hole-exploratory behavior in hole-board presents a complex microstructure where the interrelationships among its components play an important role. The multivariate approach, synergically used with traditional descriptive methods (such as evaluation of per cent distributions and latencies) offers an innovative tool to assess, at microstructural level, even subtle behavioral changes induced by manipulations of animal anxiety condition. Further studies are in progress in our laboratories to assess possible effects of diazepam, a reference anxiolytic drug, on the behavioral microstructure of rat's response to anxiety.

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