Chapter 12

Application of T-Pattern Analysis in the Study of Rodent Behavior: Methodological and Experimental Highlights

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Abstract

In our laboratories we use T-pattern analysis to study rat behavior in different and well-known experimental assays widely employed as rodent models of anxiety: the open field, the hole board and the elevated plus maze. By using Theme software and T-pattern analysis, we have observed that numerous events, characterizing rodent behavior in each experimental model, occurred sequentially and with significant constraints on the interval lengths separating them. In this chapter, for each test, we highlight some key aspects of our behavioral analyses, with a twofold attempt: first to provide the researcher with useful information concerning the application of T-pattern analysis in the study of rodent behavior and, second, to present and discuss various results of our studies.

Key words Multivariate analysis, T-pattern analysis, Anxiety, Rat, Open field, Hole board, Elevated plus maze

1 Introduction

The first step in the experimental study of human or animal behavior is commonly represented by the construction of a formal list containing descriptions of individual behavioral components. Such a formal list, namely an ethogram, dismounts the observed behavior

This chapter is dedicated to the memory of my father Prof. Giuseppe Casarrubea (March 4th 1946—June 7th 2015), historian and writer. His meticulous and patient work has clarified numerous and obscure aspects in the modern history of Italy.

As historian, he was fascinated by concepts and theories concerning T-patterns and the related possibility to study repetition of events from a scientific point of view.

As father, he would have been happy to read this book and proud to see his son Coeditor with such prestigious Colleagues and Authors.

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into discrete components that, in turn, can be characterized by means of latencies, durations, per cent distributions, and so on. In other terms, by using a quantitative approach, each component of a given behavioral repertoire can be quantified and described with numbers. In the analysis of behavior, quantitative evaluations are useful because changes of specific parameters do often provide valuable information; for instance, it could be useful to appreciate the frequency of a specific behavioral element and its modifications following the administration of a drug. On the other hand it goes without saying that the behavior of a living being is much more than simple frequencies, latencies, or durations of individual elements, disjointed from the comprehensive behavioral structure: the meaning of the behavior lies in the relationships among its constitutive components [1, 2]. As a consequence, a thoughtful approach to the study of behavior should take into consideration suitable analytical tools able to assess these relationships. Such a crucial aspect calls for different means of detection, data handling, and analysis.

The terms "multivariate analyses" are used to indicate a set of techniques aimed at the assessment of data sets with more than one variable. These methods were greatly developed only along the last three decades because they often require the computational support of modern computers and specific software. The great advantage of a multivariate approach is the possibility to assess the behavior in terms of underlying interrelationships among the behavioral elements. In addition, all multivariate techniques share the possibility to describe behavioral dynamics otherwise undetectable by means of quantitative assessments. On the basis of these features, multivariate analyses have been considered essential tools to study the structure of animal behavior in several experimental assays such as the hot plate [3–6], the open field [7], the hole board [8–10], the elevated plus maze test [11, 12], or the forced swimming test [13].

Different multivariate approaches are available. For instance, cluster analysis, stochastic analysis, or adjusted residuals analysis are multivariate techniques based on the elaboration of transition matrices. A characteristic of these methods is that they explore the comprehensive observational time window providing little information on the temporal structure of the behavior. Actually, the lack of information concerning the temporal characteristics is a common aspect of various multivariate analyses applied to the experimental study of behavior. To fill this gap the T-pattern analysis can be used. Such a multivariate technique has been developed to determine whether two or more behavioral events occur sequentially and with statistically significant time intervals [14, 15]. T-pattern analysis has been successfully used to study behavioral modifications in neuro-psychiatric diseases [16], route-tracing stereotypy in mice [17], interaction between human subjects and animals or artificial agents [18], hormonal-behavioral interactions [19], feeding behavior in broilers [20], patterns of behavior associated with emesis [21] and, in our laboratories, to investigate exploration and anxiety-related behaviors in rodents [2, 22–24]. The present article will discuss the application of T-pattern analysis in the study of rodent's anxiety-related behaviors in three different behavioral assays. Various aspects of our behavioral analyses will be highlighted with a twofold aim: first to provide the reader with methodological information concerning the application of T-pattern analysis in the study of rodent behavior and, second, to discuss various results of our studies.

2 Methods

2.1 Apparatus

In the present chapter the T-pattern analysis has been carried out to assess rodent's behavior in three different experimental assays: the open field, the hole board, and the elevated plus maze. Common characteristics of these tests are the relatively low cost and simple testing procedures.

The *Open Field* (OF) needs little introduction being the most used experimental assay in laboratories of behavioral sciences and animal psychology. This experimental apparatus generally consists of an enclosed circular, square, or rectangular perimeter where freely moving rodents are observed for a limited period of time. The OF is commonly employed to study exploration [25] and anxiety-related behaviors [26, 27]. The rationale supporting the utilization of OF in the study of anxiety lies in the natural rodents' aversion for novel environments and unprotected areas. Indeed, once placed in the OF, rats spontaneously prefer the periphery, remaining close to the surrounding walls (a phenomenon known as "thigmotaxis"). Increase of time spent in the central zone as well as the increase of the ratio central/total locomotion or the decrease of the latency to enter the central zone represent widely accepted indexes of anxiolysis [26, 27]. The OF used in the present study consisted of a square arena 50×50 cm made of white opaque Plexiglas floor surrounded by three white opaque walls and a front transparent one.

The *Hole Board* (HB), similarly to the OF, is an exploration-based assay commonly used to examine various features of anxiety-related behaviors in rodents [8, 28–34]. This experimental apparatus consists of a square or rectangular arena with a variable number of holes in the ground [28, 35, 36] where a rat (or a mouse) can insert its head. Excluding modified HBs [37], the presence of the holes represents the essential difference between an OF and a HB. Changes of head-dipping behavior (frequency, latency, duration) reflect the anxiogenic and/or anxiolytic state of animals: anxiogenic drugs decrease both the number and duration of head-dips [38], on the contrary, if anxiolytic drugs are administered, increases in the number and duration of head-dips are observed [38]. The HB used in the present study consisted of a square 50 × 50 cm arena made of white opaque Plexiglas with a raised floor positioned 5 cm above a white opaque Plexiglas subfloor and containing four equidistant holes, 4 cm in diameter. Each hole center was 10 cm from the two nearest walls so that holes were equidistant from adjacent corners. The arena was surrounded by three white opaque Plexiglas walls and a front transparent one.

The *Elevated Plus Maze* (EPM), introduced by Handley and Mithani [39], is a widely used model to assess anxiety-related behaviors in rodents. Basically, the apparatus consists of an elevated plus-shaped platform characterized by the presence of two open and two enclosed arms. EPM usefulness has spread towards the understanding of the biological basis of emotionality related to learning and memory, hormones, addiction, and withdrawal [40]. The rationale underlying the utilization of EPM in the study of rodents' anxiety-related behaviors is based on the assumption that rodents exposed to the apparatus will respond to a conflict elicited by the presence of safe parts of the maze that are closed and protected, and aversive parts of the maze that are open, unprotected, and more brightly lit [40]. The apparatus we used was elevated at a height of 50 cm above the floor [24, 41]. The closed arms were surrounded by a 50 cm wall while open arms presented 0.5 cm edges in order to maximize open-arm entries [42]. The floor of the maze was covered with grey plastic.

2.2 Subjects

Observations have been carried out on 30 specific pathogen-free male Wistar rats divided in three groups. Each group, encompassing 10 animals, was utilized for the observations in one experimental apparatus. All subjects were housed in a thermoregulated room, maintained at constant temperature. In their home cages all animals had free access to food (standard laboratory pellets) and water.

2.3 Experimental Procedures

To minimize transfer effects and avoid possible visual or olfactory influences, rats were transferred from housing room to testing room inside their own home cages and allowed to acclimate for 30 min far from the experimental apparatus. Environmental temperature in testing room was maintained equal to the temperature measured in the housing room. Concerning the OF and the HB, each rat was placed in the center of the arena and allowed to freely explore for 5 and 10 min respectively. Concerning the EPM, each rat was placed in the central platform facing an open arm and allowed to freely explore for 5 min.

All rats, experimentally naïve, were observed only once. After an observation, the apparatus was cleaned with ethylic alcohol to remove possible scent cues left by the animal. Rodents' behavior was recorded through a digital camera, and video files were stored in a personal computer for following analyses. Concerning HB and

OF the camera was placed in front of the apparatus. As to EPM the camera was placed above the apparatus.

2.4 Ethical Statement

All efforts have been carried out to minimize the number of animals used. All the experiments here described have been conducted in accordance with the European Communities Council Directive 86/609/EEC concerning the care and use of animals for scientific purposes.

2.5 Ethogram and Coding

In the present research behavioral observations have been carried out on the basis of the ethograms we have employed in our recent studies [2, 22–24]. From a methodological point of view it is important to underline that establishing an ethogram is always a critical moment because an error (e.g. a behavioral element not described or, worst, misinterpreted) is potentially able to negatively influence the comprehensive analysis. Such a statement might appear exaggerated until one does not consider that the "raison d'être" of a multivariate behavioral analysis lies in its ability to describe interrelationships among individual components. Notably, this is even more important if the multivariate approach used is the T-pattern analysis: actually, an event can be uncommon (e.g. occurring only few times for each subject and/or not in all subjects) nonetheless the temporal relationships it establishes can be extremely important for the behavioral architecture. However, once video files have been collected and the ethogram is ready/available, the following step is normally represented by the coding process, i.e., the utilization of specific software that allows the researcher to record the occurrences of all the behavioral elements performed by the actor. The result of the coding process is an event log file that is, in its simplest form, a text file containing a sequence of behavioral events occurring at specific time points (milliseconds, seconds or, even, video frames). In the present study all video files have been coded using The Observer (Noldus Information Technology BV, The Netherlands).

2.6 Search Procedure

T-pattern analysis can be carried out by means of Theme™ software (PatternVision Ltd, Iceland; Noldus Information Technology BV, The Netherlands). This software, by means of a sophisticated detection algorithm, processes event log files evaluating possible significant relationships among the events in the course of time [14]. The search advances following a bottom-up process. In brief, being A, B, C three hypothetical events occurring in a given event log file, the algorithm compares the distributions of each pair of the behavioral elements A and B searching for a time window after A such that more occurrences of A contain B than expected by chance. In this case A and B are indicated as (A B) and form a T-pattern. After that, such first level T-patterns are marked and considered as potential A or B terms in higher patterns, for example, ((A B) C). Thus, more complex patterns may be created, step by step, following this bottom-up detection process. The search is completed when no more patterns are found. More details concerning theories and concepts behind T-pattern analysis can be found in various chapters of this book and/or in our previous articles [14, 15, 22–24].

2.7 Search Parameters

To perform a search for T-patterns, Theme™ requires specific parameters. Crucial is the "significance level" (i.e. the maximum accepted probability of any critical interval relationship to occur by chance). Extremely small values of this parameter (e.g., $p = 0.0000001$) are often useless because will lead to the detection of very few and short patterns (that is, for instance, T-patterns encompassing only two events) or, more probably, no patterns at all. On the contrary, higher values (e.g. 0.05) may produce many more and longer patterns. Thus the selected significance level strictly depends on the available data that need to be analyzed. In the analysis of rodent behavior we've found that values of 0.0001 and 0.005 work very well. Additional and important parameters with a substantial impact in the detection of T-patterns are the "minimum occurrences" (i.e. the minimum number of times a T-pattern must occur to be detected), the "lumping factor" (i.e. forward and backward transition probability above which A and B of a T-pattern (A B) are lumped, that is, A and B are not considered separately but only as the (A B) pattern) and the "minimum samples" (i.e. the minimum percent of subjects in which a pattern must occur to be detected). It is important to remember that the "minimum samples" parameter has a particular relevance when samples have been concatenated. Indeed, Theme™ is able to concatenate all event log files into a single file. Such a joining procedure is very useful because it makes possible the detection of patterns that may occur only once in each event log-file and/or, possibly, not in all samples. After the concatenation of individual log files, by setting the appropriate value in "minimum samples", uncommon but possibly interesting patterns (non detectable by analyzing each individual log file) may be detected. On this subject, since behavioral observations with rodents are normally carried out with a reasonable number of subjects (e.g. 10 or 15 rats per group) it is clear that the coding process will produce a certain number of event log files that can be concatenated to search for uncommon patterns. In the present research, coherently with our previous studies [2, 22–24], the following search parameters have been employed:

- "Significance level" = 0.0001 (0.005 in OF observations)
- "Lumping factor" = 0.90
- "Minimum samples" = 50 % (100 % in EPM observations)

2.8 Statistics

Albeit each critical interval implies the existence of a statistical significance, the enormous number of possibilities of such relationships in data with several occurrences of behavioral events might raise the question whether the detected T-patterns are there only by chance. Theme™ deals with this important issue by randomizing and analyzing the original data: using the same search parameters as with the real data, the average number of patterns detected in the randomized data is then compared with that obtained from the original data. Such a randomization process is essential because if during the assessment of one or more event log files the detected T-patterns in the real data are not significantly different from the number of patterns detected in the randomized data, then it is likely that too permissive search parameters have been used and, in brief, the detected T-patterns are not at all representative of specific behavioral dynamics but, simply, have been detected only by chance.

3 Results

3.1 Quantitative Analyses

Tables 1, 2, and 3 present the ethograms used in OF, HB, and EPM respectively. Per cent distribution of behavioral elements are presented in Fig. 1. In OF (Fig. 1a) the behavioral elements more represented are immobile sniffing, walking, immobility, climbing, and front paw licking, together reaching 81.93 % of the behavior; in HB (Fig. 1b) hole exploratory activities (HD and ES) do encompass a noticeable slice of behavior and, together with walking, immobile-sniffing, and climbing, represent the 80.79 %; finally, concerning the EPM (Fig. 1c), due to the more complex ethogram used, several behavioral elements range from 1 to 10 %. However, sniffing (-Sn), walking (-Wa, -Ent), and vertical exploration (-Re, -HDip), taken together, represent more than 90 % of the total number of behavioral events.

3.2 T-Pattern Analysis

Results from T-pattern analysis demonstrated that numerous events, characterizing rodent's behavior in each experimental model, occurred sequentially and with significant constraints on the interval lengths separating them. Figure 2 presents T-patterns length distribution in open field (Fig. 2a), hole board (Fig. 2b) and elevated plus maze (Fig. 2c).

Concerning OF, 28T-patterns of different composition have been detected (Fig. 2a); as to HB, 22 different T-patterns have been detected (Fig. 2b); finally, concerning the observations in the EPM, 197T-patterns of different composition have been detected (Fig. 2c).

Figure 2 shows also the average number of patterns detected in the randomized data + 1 standard deviation (for 5 random runs).

Table 1
Ethogram of rat's behavior in the open field

Behavioral element	Description
Walking (Wa)	The rat walks around sniffing the environment
Climbing (Cl)	The rat maintains an erect posture leaning against the Plexiglas wall, usually associated with sniffing
Rearing (Re)	The rat maintains an erect posture without leaning against the wall, usually associated with sniffing
Immobile Sniffing (IS)	The rat sniffs the environment, firmly standing on the ground
Front Paw Licking (FPL)	The rat licks or grooms its forepaws
Hind Paw Licking (HPL)	The rat licks or grooms its hind paws
Face Grooming (FG)	The rat rubs its face with the forepaws
Body Grooming (BG)	The rat rubs the body combing the fur by fast movement of the incisors
Immobility (Im)	The rat maintains a fixed posture
Chewing (Ch)	The rat produces rapid jaw movements

Table 2
Ethogram of rat's behavior in the hole board

Behavioral element	Description
Walking (Wa)	The rat walks around sniffing the environment
Climbing (Cl)	The rat maintains an erect posture leaning against the Plexiglas wall, usually associated with sniffing
Rearing (Re)	The rat maintains an erect posture without leaning against the wall, usually associated with sniffing
Immobile Sniffing (IS)	The rat sniffs the environment, firmly standing on the ground
Edge Sniffing (ES)	The rat sniffs the border of the hole without inserting the head inside
Head Dip (HD)	The rat puts its head into one of the four holes
Front Paw Licking (FPL)	The rat licks or grooms its forepaws
Hind Paw Licking (HPL)	The rat licks or grooms its hind paws
Face Grooming (FG)	The rat rubs its face with the forepaws
Body Grooming (BG)	The rat rubs the body combing the fur by fast movement of the incisors
Immobility (Im)	The rat maintains a fixed posture

Table 3
Ethogram of rat's behavior in the elevated plus maze

Behavioral element	Description
Closed Arm Entry (CA-Ent)	The rat moves from the central platform to a closed arm
Open Arm Entry (OA-Ent)	The rat moves from the central platform to an open arm
Closed Arm Return (CA-Ret)	The rat from a closed arm puts its head and forepaws in the central platform then rapidly re-enters in the closed arm
Closed Arm Walk (CA-Wa)	The rat walks in a closed arm
Open Arm Walk (OA-Wa)	The rat walks in an open arm
Central Platform Entry (CP-Ent)	The rat moves from an open or a closed arm to the central platform of the maze
Immobile Sniffing (p/u-ISn)[a]	The rat sniffs the environment standing on the ground
Corner Sniffing (p/u-CSn)[a]	The rat sniffs the entrance border of a closed arm
Stretched Attend Posture (p/u-SAP)[a]	The rat stretches its head and shoulders forward
Head Dip (p/u-HDip)[b]	Scanning movements over the sides of the maze in the direction of the floor
Rearing (p/u-Re)[a]	The rat maintains an erect posture
Defecation (p/u-Def)[a]	Excrements are produced
Grooming (p/u-Gr)[a]	The rat licks/rubs its face and/or body
Paw Licking (p/u-PL)[a]	The rat licks its paws
Immobility (p/u-Imm)[a]	An immobile posture is maintained

[a]The behavioral element is considered protected (p-) if occurring in the central platform or in a closed arm, unprotected (u-) if occurring in an open arm
[b]The head dip can be protected (p-) only in the central platform, or unprotected (u-) in an open arm

In EPM, among the 24 elements of the ethogram (Table 3) only 11 elements are encompassed in the structure of detected patterns: six protected elements (CA-Ent, CA-Wa, CP-Ent, p-Csn, p-ISn, p-Re) and 5 unprotected ones (OA-Ent, OA-Wa, u-Csn, u-HDip, u-ISn). In addition all the 197 patterns can be divided in three different groups on the basis of their composition: T-patterns occurring in central platform—open arms, in central platform—closed arms and in all the three zones of the EPM.

Figure 3 illustrates the tree structure and the connection diagram of three different T-patterns occurring 11, 41, and 22 times in OF, in HB and in EPM respectively. Their terminal strings are:

– ((Wa IS)(Cl Im)) (Fig. 3a)
– (ES (HD IS)) (Fig. 3b)

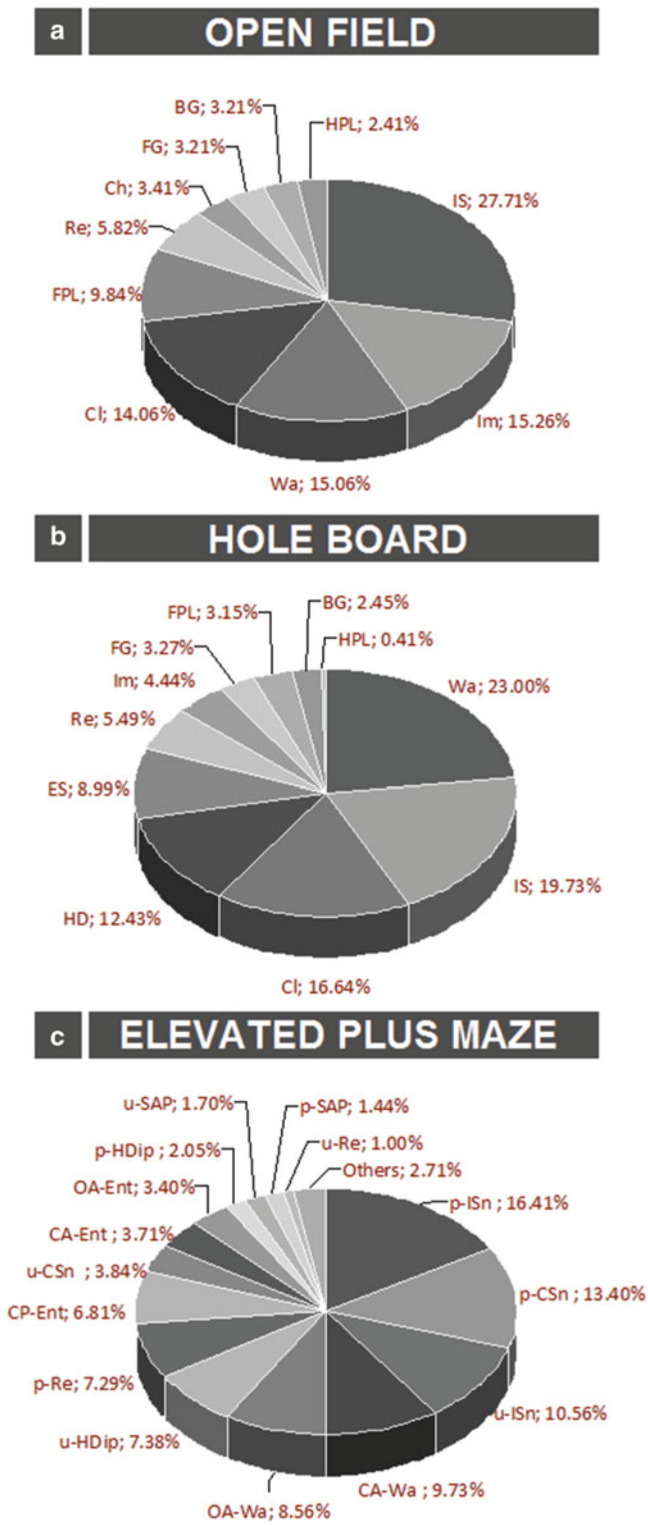

Fig. 1 Per cent distribution of behavioral elements in open field (**a**), hole board (**b**), and elevated plus maze (**c**). For abbreviations see Tables 1, 2, and 3

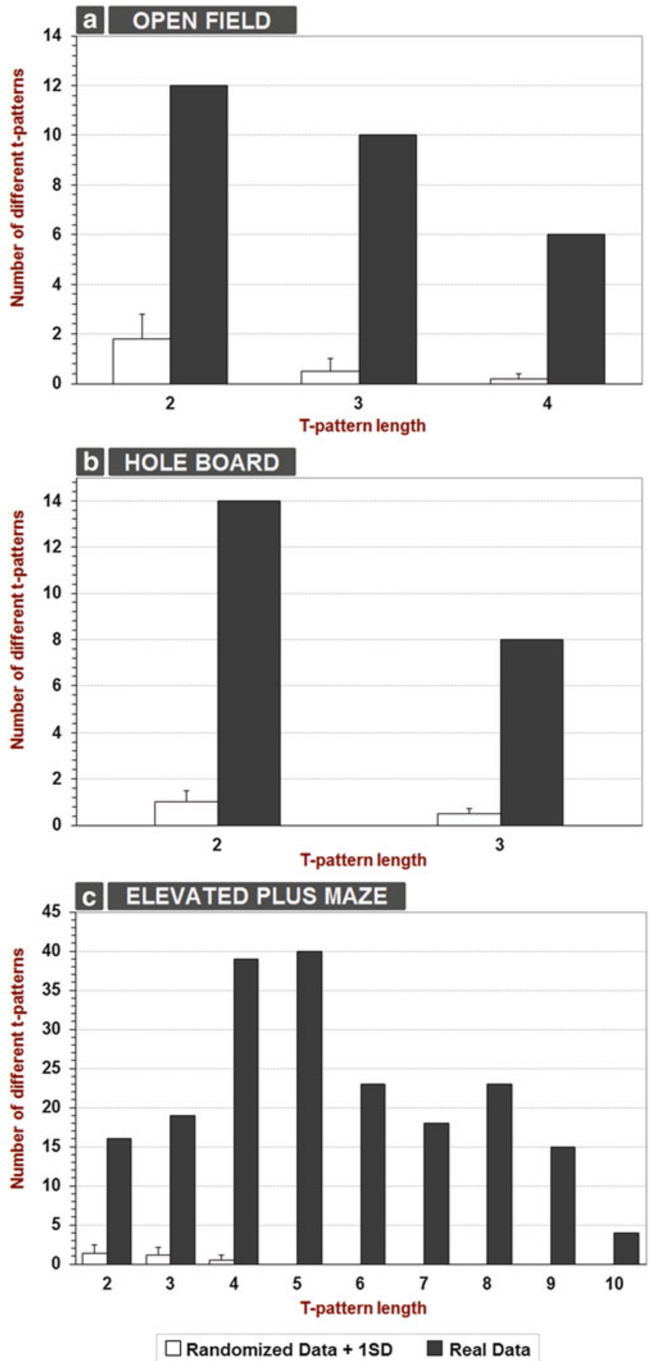

Fig. 2 T-patterns length distribution in open field (**a**), hole board (**b**), and elevated plus maze (**c**). *X*-axis = number of events encompassed in the structure of the T-pattern; *Y*-axis = number of T-patterns of different composition. *Dark columns* = real data; *White columns* = randomized data +1 SD

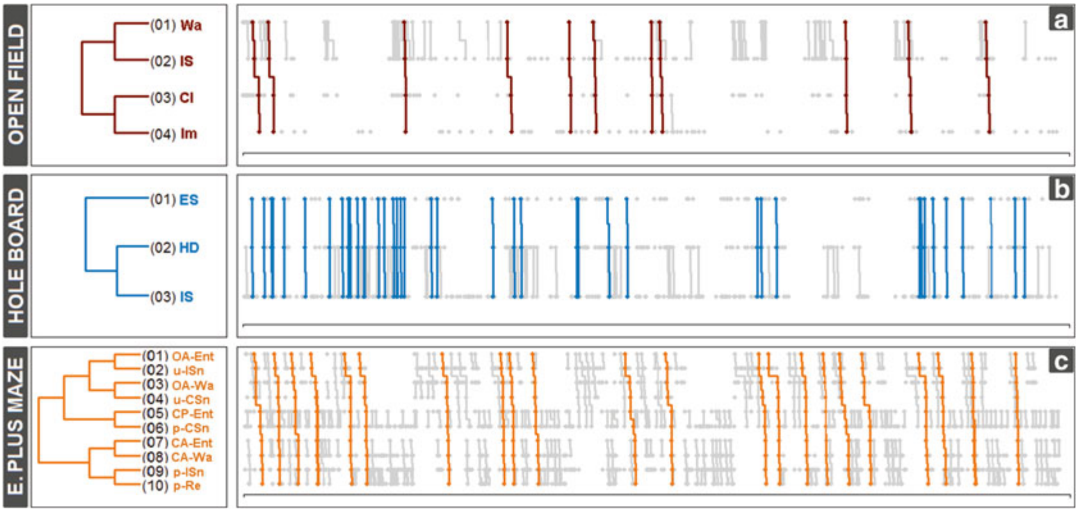

Fig. 3 Example of three T-patterns detected in open field (**a**), hole board (**b**), and elevated plus maze (**c**). *Left boxes*: tree structures. *Number in brackets* indicate the order of appearance of each event. *Right boxes*: connection diagrams. *Dots* indicate the occurrences of the corresponding events indicated in the *left boxes*. Lines connecting the *dots* represent patterns and subpatterns. Search procedure carried out on concatenated event log files, as described in Sect. 2.7. See Tables 1, 2, and 3 for abbreviations

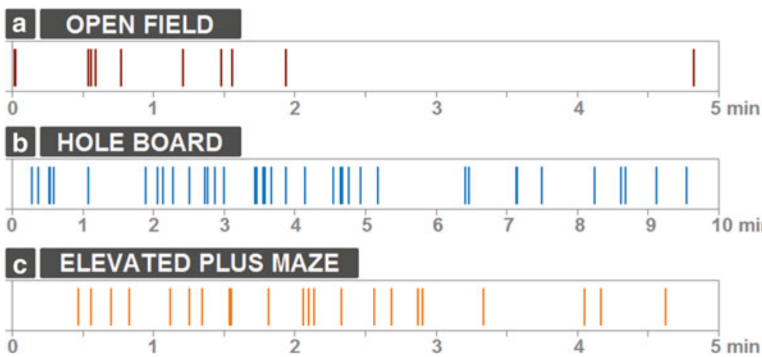

Fig. 4 Behavioral stripes of the three T-patterns illustrated in Fig. 3. *Vertical marks* indicate the onset of each T-pattern

– ((((OA-Ent u-ISn)(OA-Wa u-CSn))(CP-Ent p-CSn))((CA-Ent CA-Wa)(p-ISn p-Re))) (Fig. 3c)

The behavioral stripes of these three patterns are shown in Fig. 4.

Finally, Table 4 and Fig. 5 present the terminal strings and the behavioral stripes of 30 different patterns detected in the elevated plus maze.

Table 4
Terminal strings of 30 different T-patterns taken from the total amount of 197 detected in EPM

#	Terminal strings	A (%)	B (%)
1	(oa-wa ((u-hdip u-csn)(cp-ent oa-ent)))	100.00	0.00
2	(u-hdip (u-isn ((oa-wa u-csn) oa-ent)))	92.00	8.00
3	((u-isn ((oa-wa u-csn)(cp-ent oa-ent)))u-hdip)	90.63	9.38
4	(oa-ent (u-hdip (oa-wa u-csn)))	90.00	10.00
5	((u-hdip u-csn)(cp-ent oa-ent))	89.66	10.34
6	((u-isn ((oa-wa u-csn) oa-ent)) u-hdip)	89.66	10.34
7	((u-csn (cp-ent oa-ent))(oa-wa u-hdip))	89.47	10.53
8	((u-csn (cp-ent oa-ent)) u-hdip)	88.89	11.11
9	((oa-wa u-csn)((cp-ent oa-ent) u-hdip))	88.00	12.00
10	(u-hdip ((oa-wa u-csn)(cp-ent oa-ent)))	87.50	12.50
11	(u-hdip u-csn)	86.96	13.04
12	(((u-isn u-hdip)(oa-wa u-csn))(cp-ent oa-ent))	85.71	14.29
13	((u-isn u-hdip)((oa-wa u-csn)(cp-ent oa-ent)))	85.19	14.81
14	(((cp-ent p-csn) oa-ent)(oa-wa u-hdip))	85.19	14.81
15	(oa-ent ((u-isn u-hdip)(oa-wa u-csn)))	85.00	15.00
16	(cp-ent ca-ent)	38.27	61.73
17	((cp-ent p-csn)((ca-ent p-isn)(ca-wa p-re)))	38.10	61.90
13	(cp-ent ca-ent) p-re)	38.03	61.97
19	((cp-ent ca-ent)(p-isn p-re))	37.88	62.12
20	(p-csn ca-ent)	37.50	62.50
21	((ca-ent p-isn) p-re)	37.14	62.86
22	(ca-ent (ca-wa p-re))	36.99	63.01
23	(ca-ent ca-wa)	36.90	63.10
24	((ca-ent ca-wa)(p-isn p-re)	36.51	63.49
25	(ca-ent p-re)	36.49	63.51
26	(ca-ent (p-isn p-re))	36.23	63.77
27	((ca-ent p-isn)(ca-wa p-re))	36.17	63.83
28	(ca-ent p-isn)	35.71	64.29
29	(ca-wa p-re)	32.73	67.27
30	(p-isn p-re)	32.50	67.50

#1–#15 = T-patterns occurring in the central platform and open arms; #16–#30 = T-patterns occurring in the central platform and closed arms. %A and %B per cent distribution in the first and in the second part of the observation. For abbreviations see Table 3

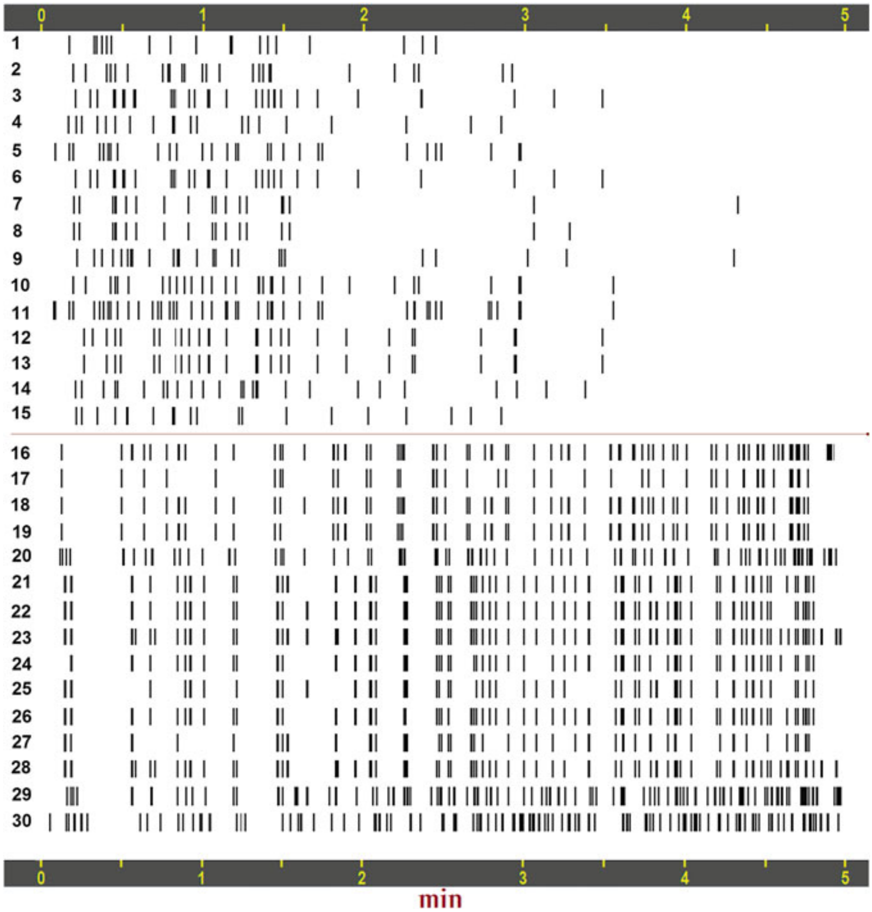

Fig. 5 Behavioral stripes of 30 different T-patterns taken from the total amount of 197 detected in EPM. Numbers on the *left* indicate the corresponding string presented in Table 4. #1—#15 = T-patterns occurring in the central—platform and open arms; #16–#30 = T-patterns occurring in the central—platform and closed arms. *Vertical marks* indicate the onset of each T-pattern

4 Discussion

Present results demonstrate that rat's behavior in OF, HB, and EPM is organized on the basis of behavioral events which occur sequentially and with significant constraints on the interval lengths separating them.

4.1 Quantitative Analyses

In the experimental study of behavior a possible synergy between quantitative analyses and multivariate approaches should always be taken into consideration. In addition it is important to consider that results from a multivariate approach might be quite difficult to interpret, even for an experienced researcher, without a preliminary outline of the studied behavior. Such a preliminary outline can be provided by means of "conventional" evaluations such as the assessment of latencies, durations, frequencies, per cent distributions etc. In this chapter we have presented, for illustrative purposes, per cent distributions of the behavioral elements. Various simple information can be appreciated: for instance, sniffing and walking activities, both in OF, HB and EPM, do encompass the largest extent of the behavioral repertoire (Fig. 1). This is not a surprising result since these activities are essential for the environmental exploration and it is well known that rodents have the innate tendency to explore novel environments/objects remaining, at the same time, as protected as possible [43]. Of course, sniffing and walking must be analyzed and interpreted taking into consideration the experimental protocol. It is also interesting to notice the peculiar distribution of grooming- and immobility-related activities, more infrequently observed in EPM than in OF or HB (Fig. 1): since these behavioral elements do require, to be performed, a fixed position, it is possible to suggest that naïve rats in the EPM have a behavioral repertoire more heavily oriented toward locomotion and exploration if a comparison with results from the open field and the hole board is carried out. Hence, on the basis of relatively simple observations of quantitative results (per cent values in this case), it is possible to provide a general outline of what a following multivariate approach may be able to better illustrate in terms of underlying behavioral dynamics.

4.2 T-Pattern Analyses

Results from Theme™ provide various interesting topics of discussion. First of all a comparison of the distribution of T-patterns on the basis of their length (Fig. 2) shows that 28 and 22 different T-patterns have been detected in OF and HB respectively (Fig. 2a, b). Concerning the EPM, Fig. 2c illustrates that 197 different patterns are present and that more complex patterns are also more numerous. Such results gain even more emphasis taking into consideration the more rigid values used for the search parameters in EPM (namely, a search run carried out using a minimum samples of 100 %). These data demonstrate a higher complexity of the temporal structure of rodent's behavior in the EPM if compared with OF or HB. The reason could be the different impact of the EPM, in comparison with OF and HB, in terms of risk assessment and approach-avoidance conflict. Actually, the presence, in the EPM, of different zones (i.e. open arms, closed arms, and central platform), characterized by different levels of aversion [40], makes this apparatus quite different from other assays utilized to study anxiety-related behavior. For instance, during an open field or a hole board test, the rodent explores first the perimeter and only in a second moment the central zones of the arena [44, 45]. Therefore, early during the exploration of an open field, the rat collects adequate information concerning, at least, the boundaries of the novel environment. In EPM, due to its structural features, all visual and somatosensory cues, originating from the different zones of the apparatus, cannot be readily accessible. It is clear that the interaction of the animal is possible only with the surrounding environment while, at the same time, other parts/zones of the apparatus remain unapproachable and will be explored when physically reachable. On the basis of these considerations, it is possible to hypothesize that the higher structural complexity of the EPM, necessarily limiting the rodent in specific zones, elicits more complex and structured behavioral patterns arising from the interaction of the subject with each zone. Coherently, all the 197 patterns detected in EPM are organized in three different groups on the basis of their composition: T-patterns occurring in central platform—open arms, in central platform—closed arms and in all the three zones of the EPM. The exemplificative T-pattern illustrated in Fig. 3c highlights such a third circumstance. In addition even at a first glance it is clear that this pattern encompasses three different subpatterns, each occurring in one of the three zones of the maze: ((OA-Ent u-Isn)(OA-Wa u-CSn)), (CP-Ent p-CSn), and ((CA-Ent CA-Wa)(p-ISn p-Re)).

4.3 T-Patterns' Stripes

The classical tree representations and the connection diagrams (Fig. 3) have the great advantage to show the structure of the patterns detected and their distribution along the observational window; moreover, these illustrative approaches are very intuitive. The drawback is the huge amount of space required. For instance, concerning present results (see Sect. 3.2), the representation of all the different patterns detected in OF, in HB and in EPM by means of tree structures and connection diagrams would be very difficult. Actually, the detection of large amount of different T-patterns each occurring even hundreds of times is not uncommon [22–24]. Last but not least, if a concatenation procedure has been carried out (see Sect. 2.7), the resulting connection diagram concerns all the concatenated log files. For these reasons we have developed the representation of T-patterns by means of behavioral stripes, that is, the illustration of the onset of each T-pattern, along the *x*-axis timeline, by means of vertical marks [22–24]. An example of this representation is illustrated in Fig. 4.

To avoid misunderstandings, it is important to underline that each mark is not an individual behavioral element but the first event of a given T-pattern. So, taking into consideration Fig. 4, each vertical mark indicates the onset of the patterns ((Wa IS) (Cl Im)), (ES (HD IS)), and ((((OA-Ent u-ISn)(OA-Wa u-CSn))(CP-Ent p-CSn))((CA-Ent CA-Wa)(p-ISn p-Re))) illustrated in Fig. 3. For clarity and completeness, the stripes should be partnered with information concerning the structure of each occurring pattern. We suggest the utilization of a separate table containing the corresponding terminal strings. Data concerning the onset of detected patterns and the terminal strings can be obtained by using the appropriate saving/export options available in Theme™. Figure 5 illustrates the onset of 30T-patterns among the 197 detected in EPM. The composition of each pattern is presented in Table 4 by means of corresponding terminal strings.

The strings in Table 4 and the stripes in Fig. 5 show 15T-patterns occurring in the central platform + open arms (from #1 to #15) and 15T-patterns occurring in the central platform + closed arms (from #16 to #30). Notably, T-patterns from #1 to 15 do occur for the largest extent within the first part of the observation; on the other hand, T-patterns from #16 to 30 have a more homogeneous distribution but with a prevalence during the second part of the test. On the basis of these results it is possible to conclude that the structure of rat behavior in the EPM has a complex temporal organization dependent on the zone of the maze explored and, importantly, on the moment of the exploration. Since it is very well known that naïve rodents in novel environments have the strong innate tendency to avoid open and illuminated areas, the presence of numerous T-patterns in central platform—open arms during the first part of the observation could be explained by a fear-related urgency to find an escape route rather than by a simple curiosity-related exploration [41].

5 Conclusion

The behavior is much more than simple latencies, durations, and per cent distributions of behavioral elements disjointed from the comprehensive behavioral structure. A given behavioral repertoire, in its natural completeness, can be literally dismounted into single pieces, namely the behavioral units of a given ethogram. Of course this is an obligatory step if a behavioral analysis must be carried out. On the other hand, if only a quantitative approach is used, the "risk" is to overemphasize each behavioral element in its individuality. It is our contention that the possibility to reduce a behavior into single "pieces", describing each individual element through even thousand of numbers does not imply the possibility to use those numbers to reconstruct the behavior and/or to figure out what the behavior is in its wholeness. If by means of suitable approaches, such as multivariate analyses, all the behavioral elements are studied in terms of their reciprocal relationships, new behavioral phenomena, otherwise undetectable, could emerge. In this chapter, by means of the multivariate T-pattern analysis, we have demonstrated, in three different and well-known experimental assays, the existence of significant patterning among the behavioral elements in the course of time. From a temporal point of view, it has been demonstrated that rodents' behavior has more complex and structured features in the elevated plus maze than in open field and/or in hole board. Such a higher complexity has been suggested to be linked with the different impact of the plus maze in terms of risk assessment and approach-avoidance conflict. In addition several methodological highlights, concerning the application of T-pattern analysis in the study of rodent behavior, have been presented.

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