# Temporal structure of rat behavior in the social interaction test

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## Introduction

The social interaction test is an important tool to study anxiety in rodents. Basically, the rationale of this test is that an increase of the interaction is indicative of a reduced anxiety; conversely, a decreased interaction indicates an increased anxiety [6]. Thus, it is not surprising that a common application of the social interaction test is the evaluation of the behavioral effects induced by anxiety modulators. However, despite the high number of studies in which the social interaction test is used, surprisingly scanty data are available on the temporal structure of the behavior of two interacting rodents. On this subject, several interesting questions and topics of discussion still remain unanswered. For instance: whether the behavior of the pair has a temporal organization, that is, if specific behavioral sequences occur during the interaction of the two subjects; second, if a temporal organization exists, could it be possible to discuss such an organization in ethological terms? Could it be possible to identify behavioral sequences with a specific functional meaning? This study has been designed to shed light on the aforementioned questions. To this aim, a refined analysis of the temporal features of the behavior, known as t-pattern analysis, has been applied. This analysis is a multivariate approach developed to determine whether two or more behavioral events occur sequentially and with significant constraints on the interval lengths separating them [10].

## Method

Eighteen male Wistar rats, two months old were used. Animals were housed in a thermo-regulated room maintained at constant temperature (23±1 °C), under a 12 h light/dark cycle (lights on = 07:00 a.m.; lights off = 07:00 p.m.). Food pellets and water were provided ad libitum. Each subject has been individually housed for 5 days before testing [5] [6] [7]. On the test day, to minimize transfer effects and avoid possible visual or olfactory influences, two rats, randomly taken from housing room, were transferred inside their own home cages to testing room 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. All rats, experimentally naïve, were observed only once. Each pair was observed for 15 minutes in a 50 x 50 cm open field apparatus. The behavior was recorded by means of a digital video-camera and video-files stored in a backup device for following analyses. Table 1 presents the utilized ethogram, arranged on the basis of previous studies of different Authors [11] [13] [14] [15]. All video files were coded using The Observer (Noldus Information Technology, The Netherlands).

**Table 1.** Ethogram of rat behavior in the social interaction test.

| Intra-subject | Abbr. | 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. |
| 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 | FGr | The rat rubs its face with the forepaws |
| Body Grooming | BGr | The rat rubs the body combing the fur by fast movement of the incisors |
| Shaking | Sh | The rat shakes its head and body with rapid semicircular movements |
| Immobility/Resting | Imm | The rat maintains a fixed posture |
| Immobile Sniffing | ISn | The rat sniffs the environment standing on the ground |
| **Inter-subject** | **Abbr.** | **Description** |
| Withdrawing | Wit | One rat walks or runs away from the other rat. |
| Following | Fol | One rat follows the partner while the other is walking away |
| Approaching | App | One rat walks in the direction of the partner, while the other rat is immobile or is alredy approaching him |
| Crawling over | CrO | One rat walks over the partner |
| Crawling under | CrU | One rat walks under the partner |
| Boxing/Wrestling | Box | Offensive/aggressive behaviors such as pawing, pouncing, nosing, biting, boxing, kicking, wrestling |
| Leaning on | LeO | One of the rats leans with its forelimbs on the other rat that, in turn, maintains all the four paws on the ground. |
| On-top | Top | One of the animals stands over the partner that lies with its back on the floor |
| On-back | Bck | One of the animals lies with its back on the floor with the other animal standing over it |
| Mounting | Mnt | One of the rats holds the other rat's trunk with the forelimbs |
| Social grooming | SoG | One of the rats grooms the partner's body, neck or face |
| Social sniffing | SoS | One of the rats sniffs the partner's face and/or body |
| Ano-genital sniffing | GeS | One of the rats sniffs the partner's anogenital area |

To assess the temporal relationships among behavioral events, event log files, obtained from coding process, were analyzed by means of the software Theme (Noldus Information Technology bv, The Netherlands; Patternvision Ltd, Iceland). The software can analyze any time-based data; on this subject it is important to mention that the time unit can be of any size (e.g. seconds, milliseconds, days, years), depending on the system being studied. Theme's detection algorithm searches for statistically significant relationships between events in behavioral data, by taking into account the order, timing, and frequency of these events [10]. In brief, given an observational period T0-Tx where several behavioral events occur, the algorithm compares the distributions of each pair of the behavioral elements "A" and "B" searching for an interval so that, more often than chance expectation, A is followed by B within that time window. In this case A and B do represent a two-element t-pattern, indicated with the string (A B). In a second step, such first level t-patterns are considered as potential terms in higher patterns, for example, ((A B) C) and so on. Thus, following this hierarchical bottom-up detection process, more complex patterns may be identified up to any level. Before a t-pattern search is performed, the software requires specific search parameters which, in the present study, are: "significance level" (maximum accepted probability of any critical interval relationship to occur by chance) = 0.0001; "lumping factor" (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 = 0.90; "minimum samples" percent of subjects in which a pattern must occur to be detected) = 100; "minimum occurrences" (minimum number of times a t-pattern must occur to be detected) = 9. Such parameters were selected to obtain the identification of behavioral sequences that were present in all the samples and were characterized by high level of significance. More exhaustive descriptions of concepts, theories and methodological approaches underlying t-pattern analysis can be found in previous works [1] [2] [3] [10].

## Results

221 different t-patterns have been detected. On the basis of the quality of the events present in the structure of these patterns (namely, intra- or inter-subject events, see tab. 1), four different categories have been identified (fig. 1): a first category consists of t-patterns containing exclusively inter-subject events; a second one consists of t-patterns containing both inter- and intra-subject events; a third category encompasses patterns with events performed both by subject 1 and subject 2 as well; finally, a category consists of patterns with events performed by one of the two subjects. Figures 2-5 illustrate the terminal strings and the occurrences/onset of the most complex and frequent t-pattern for each category.

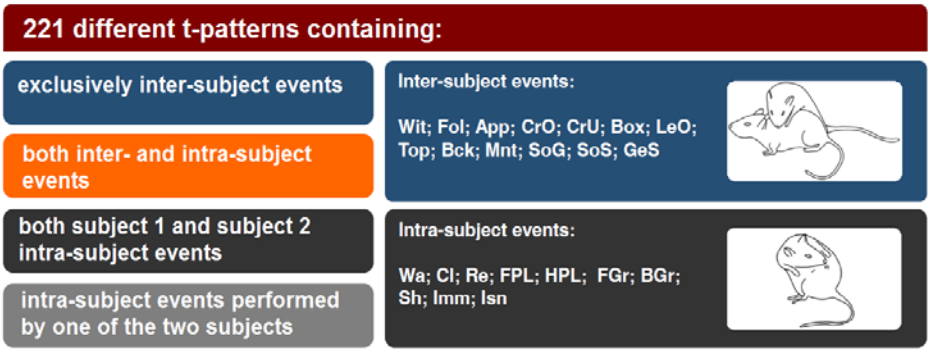


**Figure 1.** T-pattern analysis has revealed 221 different t-patterns. Left: four different categories of t-patterns; right: structural events (inter- and intra-subject) from the ethogram. For abbreviations see tab. 1.

## Discussion

Multivariate analysis has revealed four different sets of t-patterns (figure 1): t-patterns where the sequence consists exclusively of structural components defined on the basis of the relationships with the partner, namely, inter-subject components (figure 1 and figure 2). A t-pattern encompassing only this type of events is indicative of the interaction between the two tested subjects.

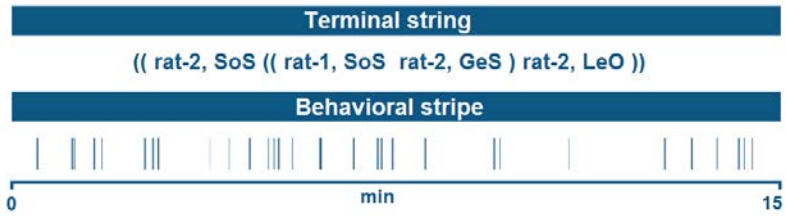


**Figure 2.** Terminal string and behavioral stripe of the most complex and frequent t-pattern for the category containing exclusively inter-subject events. For abbreviations see tab. 1.

A second category of t-patterns encompasses sequences consisting both of inter- and intra-subject events (figure 1 and figure 3). For the definition of what a t-pattern is (namely, a statistically significant sequence of events), all the patterns containing inter-subject components do represent an interaction as well. Our analysis has also detected various t-patterns consisting only of intra-subject events. Such an outcome calls for an interesting question/topic of discussion: is it correct to consider a t-pattern containing intra-subject events, carried out by the two rats, representative of an interaction? For instance, does the sequence ((rat-2,Wa rat-2,Cl)(rat-1,ISn rat-1,Wa)) illustrated in figure 4 represent an interaction?

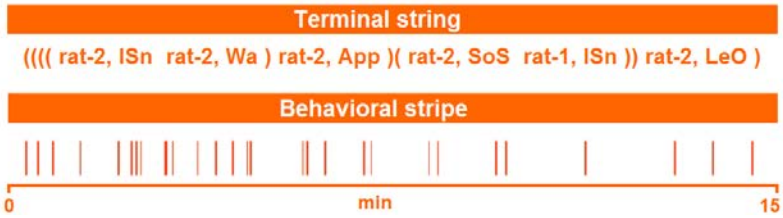


**Figure 3.** Terminal string and behavioral stripe of the most complex and frequent t-pattern for the category consisting both of inter- and intra-subjects events. For abbreviations see tab. 1.

The answer should be affirmative because such a t-pattern reveals a clear-cut causality between the behaviors of the two subjects, namely, following Walking (Wa) and Climbing (Cl) produced by the rat-2, rat 1 produces

Immobile-Sniffing (ISn) and Walking (Wa); importantly, such a succession of events is significantly repeated several times along the 0 - 15 min observational period, as clearly illustrated by the corresponding stripe (figure 4).

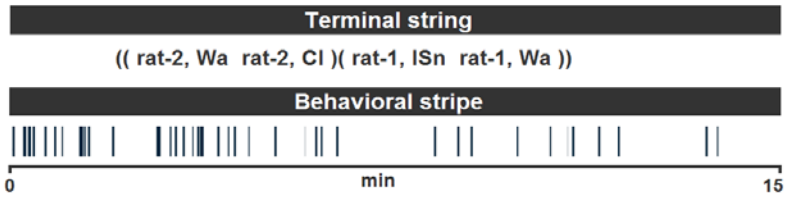


**Figure 4.** Terminal string and behavioral stripe of the most complex and frequent t-pattern for the category containing intra-subject events carried out by the two rodents. For abbreviations see tab. 1.

Finally, it has been possible to identify a fourth set of t-patterns containing intra-subject events produced by one of the two animals. In this context, the interaction is absent because the rat carries out a behavioral sequence consisting of events totally disjointed from the partner both from a qualitative and temporal point of view. Figure 5 exemplifies such a fourth category: the whole sequence is carried out by rat-2 and does contain exclusively Immobile-Sniffing (ISn), Walking (Wa) and Rearing (Re), that is, intra-subject activities.

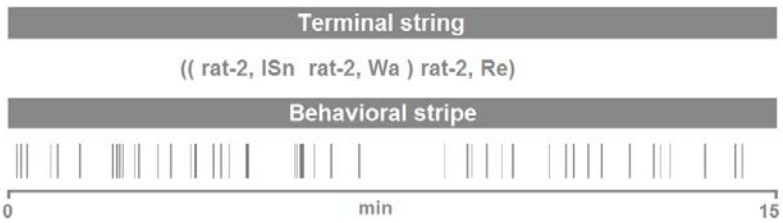


**Figure 5.** Terminal string and behavioral stripe of the most complex and frequent t-pattern for the category containing only intra-subject events carried out by one of the two subjects. For abbreviations see tab. 1.

These preliminary results demonstrate that rat behavior in the social interaction test is structured on the basis of several recurring sequences of events characterized by statistically significant constraints on the interval lengths separating them. The multivariate t-pattern analysis could represent a valid approach to study hidden characteristics of rodents social behavior; an example, on this subject, is represented by the detection of t-patterns containing only intra-subject components where a clear, otherwise undetectable, causality in the behavioral sequences of the two animals can be identified. Finally, t-pattern analysis, coupled with refined approaches aimed at the automated analysis of behavior [4] [8] [9] [12], could represent a valuable tool to investigate the temporal features of social behavioral dynamics, anxiety-related behaviors and, as a consequence, to assess the efficacy and/or the effects induced by the administration of potential anxiety modulators.

## Ethical Statement

All efforts were made to minimize the number of animals used and their suffering. All the experimental procedures were conducted in accordance with the European Communities Council Directive (86/609/EEC) and approved by the Veterinary Committee officially appointed by the University of Palermo.

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