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Application of multivariate T-pattern analysis in the study of social interaction in rats

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Introduction

The social interaction test, introduced by File and Hyde in 1978,¹ is a well known behavioral model used to investigate anxiety-related behaviors in rodents. Basic premise of the test is that the behavior of a rodent influences the one of the other.

An increased interaction between the two subjects is indicative of a reduced anxiety condition, in contrast, a reduced interaction indicates a higher anxiety. Albeit the test has been largely used to study anxiolytic and neuroleptic drugs, no data are available on the temporal structure of the behavioral interactions. In addition, interesting questions still remain unanswered.

For instance, is it possible to identify recurring temporal sequences from the two interacting rodents? If so, do such sequences encompass specific behavioral events? Could such sequences have an ethological meaning?

Materials and Methods

In this preliminary study, four male Wistar rats (220-250 gr) were used. Each subject was housed in a thermo-regulated room. The day of the experiment, pairs of rats, randomly taken from housing room, were placed in an open field apparatus (50 x 50 cm) and observed for 15 min. The behavior of the animals was recorded with a digital

video camera and video files stored in a personal computer. Using an ethogram, obtained on the basis of previous observations,²⁻⁵ video files were coded by means of a software coder and the obtained event log files used for following analyses. Both quantitative and multivariate t-pattern analyses were carried out. The latter is a multivariate approach based on the utilization of a specific software that, by means of an advanced search algorithm, processes event log files evaluating possible significant relationships among the events in the course of time.⁶ Theories, concepts and procedures concerning such a multivariate analysis of behavior can be found in our previous articles.⁶⁻⁸

Results

The ethogram is presented in Table 1. The behavioral activities are classified taking into consideration their characteristics: *non social* (produced in absence of interactions), and *social* (produced during interactions).

Preliminary results, obtained from the analysis of two pairs of rats, are presented. Per cent distribution, evaluated both for social and non social activities are illustrated in Figure 1. ISn, Wa, Cl, Re, FPL, Imm and Sh represent 95.50% of the non social behavioral repertoire; on the other hand, SoS, App, Wit, LeO, SoG and GeS represent 84.50% of the social one. T-pattern analysis demonstrated, in both pairs of rats, the presence of significant constraints among numerous events in the course of time. Figure 2 illustrates a t-pattern detected in one pair of rats.

Discussion

The current research represents the first effort to study the temporal structure of social interaction in rats by means of multivariate t-pattern analysis. Per cent distribution (Figure 1) shows that sniffing related (ISn, Cl, Re, SoS, GeS) and walking related (Wa, App, Wit) activities (Table 1) are the most represented both in non social and social behavior.

Coherently, the t-pattern presented in Figure 2, encompassing only sniffing- and walking-related activities, well depicts the role of these behavioral events in moulding the temporal structure of the behavior. Interestingly, some kinds of behavioral symmetries were observed, where a behavior, carried out by one of the rats, was followed by the same behavior carried out by the other animal (Figure 2).

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Table 1. Ethogram. First column: behavioral element. Second column: abbreviation. Third column: description.

Non Social	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 ribs 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
Social	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 already 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 its 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

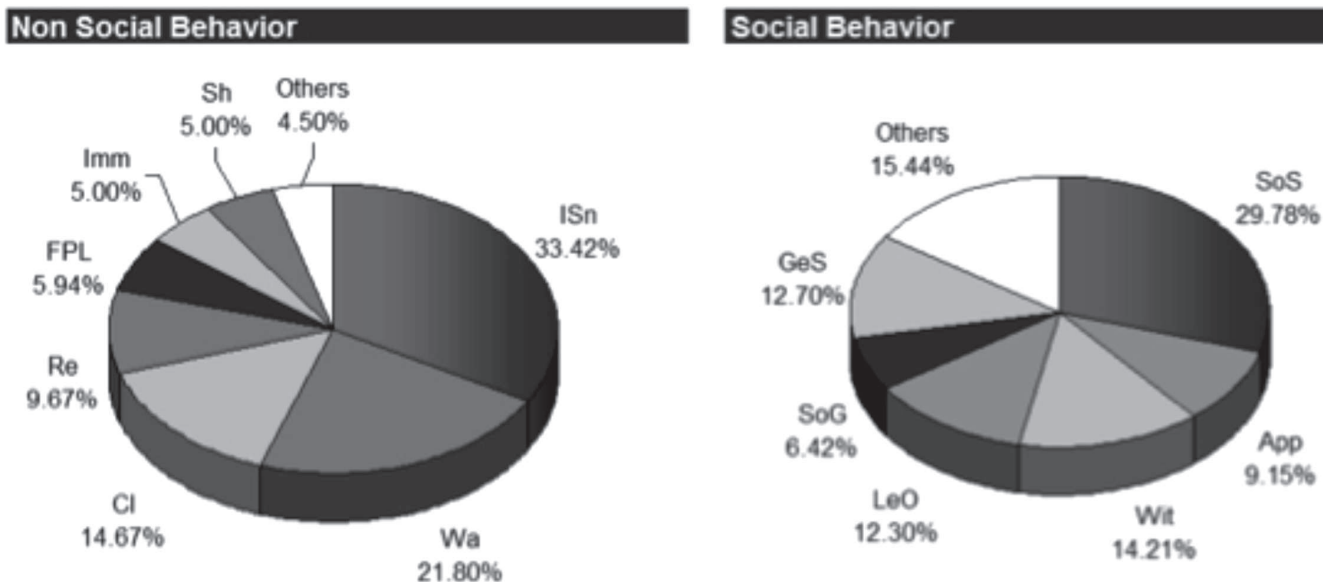


Figure 1. Per cent distribution of non social (left pie) and social (right pie) behavioral elements carried out by the rats. Others=behavioral elements <5%. For abbreviations see Table 1.

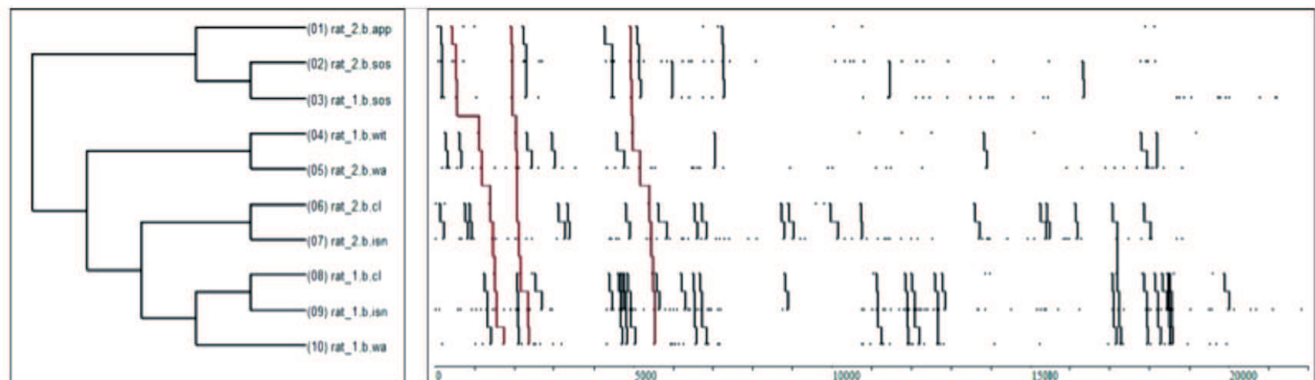


Figure 2. T-pattern detected in one of the two pairs of rats. Left box: tree structure. Number in brackets indicates the order of appearance of each event. Right box: connection diagram. Dots show the occurrences of the corresponding events reported in the left box. For abbreviations see Table 1.

Conclusions

To sum up, preliminary results obtained from t-pattern analysis demonstrate that the behavior of rats in the social interaction test is extremely complex and structured on the basis of numerous, recurring and statistically significant sequences of events.

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