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Time as the language of Behavior: events, sequences, patterns and meanings

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Abstract

The temporal structure of behavior cannot be considered a mere background condition; rather, it is a fundamental component in the organization, expression, and interpretation of the actions of all living beings. Time flow does shape how behaviors unfold, how they are perceived, and how meaning is structured from them. This paper explores the temporal dynamics of behavior by initially examining the aspects from which they originate, namely discrete events, their sequences, and patterns. Methods, instruments and approaches, ranging from software coders, to transition matrices, to T-pattern analysis, used to study these different but intrinsically related behavioral products will be discussed. In this context, particular emphasis will be placed on the crucial role of the temporal organization, in order to highlight how temporal relationships among events and the complex structural regularities underlying the detection of their patterning do define the intrinsic architecture of action and, most importantly, its meanings.

Keywords: Events, sequences, patterns, T-pattern analysis, TPA

1. Introduction

In the field of Behavioral Sciences, attention has historically been focused on individual actions, choices and responses, i.e. on what characterizes the basis of behavior (Casarrubea 2024). Understanding the “*what*” effectively represents the first essential step in any behavioral study: which *actions*, which *choices*, which *responses* has the subject under examination produced, whether human or otherwise?

However, the temporal dimension of these events can be just as revealing, if not more so. Indeed, the temporal dimension contains intrinsic and essential information such as causality, intentionality, predictability, and coherence of processes. Just as language possesses a syntax (Haspelmath 2020), behavior does exhibit its own grammar, a structure capable of providing more complex and comprehensive insights for the study of the cognitive and emotional states of the subject under examination.

The temporal dimension is intrinsically connected to physiological and behavioral processes, and more generally to life itself, in all organisms. On this subject, chronobiology has established that organisms exhibit temporal organization across multiple scales, ranging from neural timing to circadian (and even seasonal) rhythms (Teki 2016). These temporal patterns are not merely descriptive features: they reflect fundamental and general organizational principles of biological systems (Dominoni et al. 2017). Circadian rhythms, for example, driven by what may be defined as endogenous “clocks,” regulate virtually every aspect of physiology and behavior, influencing not only sleep-wake cycles but also hormonal secretion, metabolism, cognitive functions, and emotional regulation, just to name a few (De Sanctis 2017; Weil and Nelson 2014). Beyond circadian rhythms, other temporal factors also warrant attention. Ultradian rhythms, for instance, organize behavior on temporal scales shorter than 24 hours (Kembro et al. 2023, 2024; Guzman et al. 2017). Interestingly, it is well known that such rhythms are not uniform across individuals; indeed, individual differences in circadian temporal preferences generate variations in the timing of optimal performance (May and Hasher 2023). Timing mechanisms allow organisms to anticipate events and coordinate behavior in an optimal and context-sensitive manner (Antle and Silver 2009). In organizational and social contexts, temporal dynamics such as rhythm but also duration and sequence shape both individual and collective behavioral outcomes (Shipp and Cole 2015; Griep et al. 2021).

Despite these evidences, temporal factors are not infrequently overlooked in behavioral research. Such an approach may lead to increased measurement errors, reduced statistical power, failures in replication, and functional models that are fundamentally misleading or, at the very least, incomplete. In this scenario, the present paper aims to synthesize the evidence regarding the crucial role of time in the behavioral sciences, with the goal of promoting greater awareness of the evaluation of this aspect within studies in the field. Through an examination of the different manifestations of temporality and its relevance in the context of behavior, this article seeks to offer a conceptual framework designed to integrate the temporal dimension into future research

directions. In order to provide a coherent and comprehensive overview, the “*morphological*” features of each behavioral output, i.e., its phenotypic and manifest aspects, will be first discussed. These discrete components (Fig. 1) represent the fundamental elements of any behavioral analysis, representing the indispensable foundation upon which the temporal variable can be subsequently integrated.

2. Methods and instruments

In behavioral analysis, it is possible to identify three increasing levels of complexity, ranging from the evaluation of discrete events to the evaluation of sequences of such events, and finally to the evaluation of patterns, i.e., complex architectures that originate from the interaction of the basic constituent events (Fig. 1). Discrete behavioral phenomena represent the basic units, individual actions (e.g. a single blink of the eye, the pressing of a key, the head movement made by a rat, etc.) which, experimentally, can be analyzed for their frequency, duration, or latency, i.e., based on their purely quantitative characteristics. However, behavior rarely consists of isolated events unrelated to each other: when these acts occur in a non-random order, behavioral sequences emerge, where the probability of an action is influenced by the previous one, e.g., the motor chain of grooming in rodents. At a higher level of complexity, we reach the behavioral patterns: stable and organized configurations orbiting an underlying structure. These patterns are not merely the algebraic sum of acts, but reflect the synergy between internal states and environmental stimuli, allowing us to predict the evolution of an interaction or decision-making process in the long term. Studying each type of behavioral product involves very different approaches and instruments; these will be outlined in the sections below.

2.1. Methods and tools for the study of discrete behavioral events

These approaches focus on the precise evaluations of a single event and its quantitative aspects such as its frequency, durations, per cent distribution etc. All these approaches aim to capture the event with the minimum error (Tab. 1A).

The primary tools for studying discrete phenomena are undoubtedly software tools that allow for the on-screen annotation of events observed in a video file. Essentially, instead of taking notes with pencil and paper, the observer utilizes software interfaces where each key corresponds to a behavior, or selects from a pre-defined set of events using a mouse. The result is the so-called event-log file, i.e., in its simplest form, a text file containing the events and the timestamps at which they occur. These event-log files serve as the foundation for subsequent analyses. The gold standard of coding software is represented by The Observer (Noldus IT, The Netherlands). This program has evolved significantly over the years, transforming from a simple tool for annotating observed

behaviors (Noldus et al. 2000) into the versatile and comprehensive tool it is today (Noldus IT 2026a). Another notable coding software is BORIS (Behavioral Observation Research Interactive Software) (Friard and Gamba 2016); like The Observer, it allows users to upload a video and synchronize keypresses with the video's timer. Significant advantages of this tool are its continuous and frequent updates by the development team, along with its intuitive and user-friendly interface. Solomon Coder (Péter 2017) is another flexible tool characterized by an intuitive and lightweight interface; it ensures the coding of parameters such as frequency, duration, and latency of the observed activities and offers the possibility to export of data into various formats for statistical analysis. JWatcher (Blumstein and Daniel 2007) is another lightweight event-logger for quantifying discrete behaviors in sequences; available for Windows-, Linux- and MAC- based platforms, it also offers a built-in suite of statistical tools, which can be applied directly to observational data. Lastly, within the domain of coding software, Lince Plus (Soto-Fernández et al. 2022) is a versatile tool available for both PC and mobile platforms, designed for systematic observation in sports and health sciences, specifically for field-based contexts in areas such as training, education, psychology etc. That being said, in the fields of psychophysics and cognitive psychology, when the need to evaluate reaction times is crucial, standard computer hardware (e.g., commercial USB keyboards and/or keypads) can introduce an unacceptable delay, ranging between 15 and 30 ms. This delay, seemingly negligible, can invalidate results, especially if the effects under study are on the order of a few tens of ms. In such cases, high-performance button boxes or response pads are used. These instruments are specifically designed to bypass the bottlenecks of commercial hardware and, with the aid of specific software packages such as Psychtoolbox and PsychoPy, ensure greater precision in recording reaction times (Bridges et al. 2020).

Alongside software coders, which obviously require a trained/experienced human operator, automatic detection systems deserve mention. In many contexts, particularly in experimental psychology and neuroscience, physical sensors are used, such as those contained in specialized instruments like the classic Skinner Boxes and modern Operant Chambers (Soares et al. 2016). These setups are essentially cages equipped with a wide array of micro-switches (e.g., for lever pressing) or infrared sensors (e.g., for nose poking, where the animal inserts its snout into a hole). Each sensor activation is recorded with millisecond precision, allowing for the analysis of even micro-variations in force or reaction time. The last two decades have seen a progressive increase in wearable devices (e.g., wristbands and smartwatches) capable of recording discrete wrist movements via accelerometers. These devices are very useful and reliable for studying motor tics or micro-arousals during sleep. This technology, known as *actigraphy*, has revolutionized the study of human behavior outside the laboratory, transforming simple watches and wristbands into powerful psychophysical monitoring tools. While the Button Boxes and/or Response Pads mentioned earlier capture intentional responses, accelerometers in smartwatches are capable of capturing involuntary activities. These methods can be employed to study sleep and its characteristics (Morgenthaler et al. 2007). Another promising application is the study of motor tics and movement disorders, such as Tourette syndrome (Keenan et al. 2024) or Parkinson's disease (Zampogna et al. 2020). In some cases, however, where very rapid movements occur, the behavior is not easily detectable. Saccadic movements, for example, are discrete but extremely rapid eye movements. Eye-trackers capture the eye's position with remarkable precision (Kassner et al. 2014; Holmqvist and Anderson 2017). These highly refined and precise assessments allow for the identification of the exact moment when

attention shifts from point A to point B, a fundamental data point for the study of decision-making processes.

Finally, video tracking systems such as Ethovision (Noldus IT, The Netherlands) should be noted. These systems are designed to automatically detect a subject's movement, thereby allowing their position and displacement to be tracked without observer intervention (Noldus et al. 2001; Noldus IT 2026b). FaceReader (Noldus IT, The Netherlands) is an extremely powerful and versatile tool for the automatic analysis of facial expressions, including eye-tracking. It is primarily used in academic research, but also in neuromarketing and usability testing for software and web pages (Noldus IT 2026c).

2.2. Methods and tools for the study of behavioral sequences

When moving from individual acts to behavioral sequences, the focus shifts from the isolated event to the relationship of order and dependency between them. Approaches that analyze behavioral sequences (Tab. 1B), allow us to ask not only *"what happened?"* but, importantly, *"given that A occurred, how likely is B to follow?"*

Lag Sequential Analysis (LSA) is the method of choice for determining whether a behavioral sequence occurs with a frequency greater than what would be expected by chance. The Generalized Sequential Querier (GSEQ) software is the gold standard for this type of analysis (Bakeman and Quera 2011). This tool allows for the input of coded data files (often originating from the coding software discussed earlier) and the analysis of contingencies between behaviors at different *"lags"*. For example, a Lag 1 analysis examines what happens immediately after the target event; a Lag 2 analysis examines what happens two steps later.

In behavioral sequences, it is often assumed that future behavior depends on the current state. Markov Chains model these transitions. Specific R packages (such as *"markovchain"*) (R Core Team 2026) are available for these analyses. The primary mathematical tool underlying Markov chains is the transition matrix, and in particular its stochastic elaboration, which displays the probability of moving from one state to another. Another specific refinement of transition matrices involves their transformation into adjusted residual matrices. In brief, adjusted residuals do represent the difference between an observed value and an expected one (Everitt 1977); an important advantage of these matrices is that can be expressed according to a Z-distribution (so that p-values can be identified using a standard Z-distribution table) indicating transitions that occur significantly more or less often than expected (Spruijt and Gispen 1984; Casarrubea et al. 2022). Stochastic probabilities and adjusted residuals matrices can be also represented by means of different graphical representations (Spruijt and Gispen 1984; Casarrubea et al 2011, 2017). An early tool for managing transition matrices is MatMan (de Vries et al. 1993), a software tool which was available for several years as a commercial software package known as Matman v1.1 (Noldus IT, The Netherlands). Figure 2 illustrates an example of the sequential organization and probabilistic structure among five key elements of the behavioral repertoire of the rat tested in the Hole-board,

a widely validated experimental apparatus used for the assessment of anxiety-related behavior in rats and mice (Casarrubea et al., 2023).

Finally, it is important to mention that behavioral sequences can also be analyzed in terms of networks. Gephi (Bastian et al. 2009) and Cytoscape (Shannon et al. 2003) are powerful software packages used in this context. Originally developed for social network analysis, they are employed to visualize behaviors as *nodes* and transitions as *links*. Their utility lies in the fact that they allow for the identification of specific behaviors that act as bridges between different sequences and are, therefore, central to the organization of the subject's behavioral repertoire

2.3. Methods and tools for the study of behavioral patterns

Discussing tools for studying behavioral patterns means entering the most advanced level of behavioral analysis, that of hidden architectures. Accordingly, the tools and technologies for studying behavioral patterns are the most complex and generally very time-consuming to master, both conceptually and in terms of practical applications (Tab. 1C).

Versatile tools whose natural placement lies at the crossroads between the study of individual events and the search for broader patterns, with a strong inclination toward the latter, are Principal Component Analysis and Factor Analysis (Jolliffe 2002; de Winter and Dodou 2016). Widely applied in numerous fields ranging from Psychology to Software Engineering, these methods are highly effective in behavioral studies for dimensionality reduction and data synthesis. Rather than focusing on isolated acts, they can be able to identify how various acts combine to form a behavioral profile.

Pose estimation and machine learning-based methodologies are approaches through which patterns are no longer extracted solely by hand, but via algorithms that analyze the video pixel by pixel to reconstruct the subject's skeleton. In this regard, deep learning-based software such as DeepLabCut (Mathis et al. 2018) and SLEAP (Social Leap Estimates Animal Poses) (Pereira et al. 2022) allow for the high-precision tracking of joint points (e.g., head, tail, limbs) without the use of physical markers. B-SOiD (Behavioral Segmentation of IDentified points) (Hsu and Yttri 2021), in turn, employs unsupervised learning to automatically segment behavioral patterns, identifying motor characteristics that the human eye might not discern (e.g., a micro-hesitation in gait, an imperceptible head movement, etc.).

A quite distinctive approach is that of State-Space Grids. Through these, it is possible to visualize behavior as a trajectory within a "*state space*". In this regard, the GridWare software allows for the mapping of the behavior of two subjects onto a grid (Hollenstein 2013). Patterns do emerge as attractors: areas of the grid where the dyad continuously returns or where it remains stuck. It is ideal for studying the stability and flexibility of behavior over time, key concepts in clinical and developmental psychology.

Finally, T-Pattern Analysis (TPA), introduced by Magnus S. Magnusson (Magnusson 1996, 2000, 2004; Magnusson et al. 2016), which will be also discussed later, represents a solid and advanced approach for studying the temporal dynamics of behavior. Unlike LSA, which examines what follows

what, TPA allows the detection of significant temporal relationships between events, even when they are separated by long time intervals or other “*distractor*” behaviors. The core concept of T-patterns is that when an event is followed by another *within a relatively constant and statistically significant* time interval, a T-pattern is identified. Interestingly, these patterns can be hierarchical, meaning that small patterns can merge to form larger and more complex ones. [Figure 3](#) illustrates, using TPA, an example of the temporal structure underlying the relationships between the five behaviors depicted in [Fig. 2](#), in a group of rats tested in the Hole-Board.

2.4. Choosing the appropriate methods and tools: pros and cons.

The questions spontaneously arise as to whether there is a preferred method, if they can be used in synergy, and what the pros and cons of the different approaches are. Actually, choosing between a single specialized research method is a critical decision. Indeed, each path does offer specific advantages, but also inherent compromises that must be carefully considered. The choice of tool strictly depends on the underlying scientific question and the nature of the available data. For example, if the aim of the study is to measure the frequency or duration of a response, or its latency following a stimulus or drug administration, then tools suited for the study of discrete phenomena should be employed ([Tab. 1A](#)). If one wishes to study aspects related to transition probabilities, then transition matrices and related elaborations could be ideal ([Tab. 1B](#)). Finally, if there is a concrete suspicion that the observed behavior possesses a complex structure not discernible to the naked eye, or if one wishes to reveal if and how events are connected over time, then tools aimed at pattern analysis will be the necessary choice ([Tab. 1C](#)). Beyond these scientific considerations, there are other important additional factors, often closely interdependent, that must be seriously considered: available time, available funding, and, last but not least, the cost-benefit ratio.

Broadly speaking, the further one moves from left to right in [Fig. 1](#), the more demanding behavioral assessments become in terms of required resources, time commitment and economic costs. Speaking of the two extremes, indeed, while the simple counting of a specific behavioral element might require an amount of time ideally comparable to the observation window itself and very simple means (e.g., counting how many times a rat in a Hole-Board visits one of the four identical floor-holes during a 5-minute session), on the other hand, evaluating the relationships between this element and the remaining components of the behavioral repertoire can be considerably more complex (e.g. assessing the relationships among Head-Dip and all the remaining components). These analyses require methods such as matrix-based analyses, LSA or TPA which are inherently much more demanding not only in terms of hardware and software, but also in terms of time investment and more specialized expertise.

2.4.1. Discrete phenomena: frequencies, durations, and latencies

The study of discrete phenomena represents the basis of any quantitative behavioral analysis. The primary advantage of all approaches and tools aiming at the study of discrete components of the behavior lies in a resulting extraordinary objective clarity and statistical robustness. Indeed, by focusing on parameters concerning specific responses, researchers can obtain “high-resolution” numerical data easily comparable across different experimental groups. In addition, and importantly, it is the most “reproducible” approach, particularly effective when the research question is strictly causal or pharmacological. For instance, measuring the duration of head-dipping in a Hole-Board, the frequency of lever-pressing in an operant chamber or the latency to jump in a Hot-Plate provides a clear metric of the observed behavior or drug efficacy. In addition, because these events are treated as discrete units, they allow for the application of powerful statistics. Altogether these aspects ensure that the findings are highly reproducible and require smaller inferential leaps than more interpretative models.

On the other hand, the main drawback concerning the evaluation of discrete phenomena is the inherent *reductionism* of this approach. Indeed, by “*atomizing*” behavior into independent events, this method inevitably loses the sequential context. It treats a behavioral repertoire as a set of isolated points rather than a continuous flow, potentially overlooking how the meaning of a response might change depending on the actions that preceded it. Consider, for example, the grooming behavior in rodents or social interactions in primates. If we simply count the number of grooming bouts, we might miss the fact that a specific sequence (e.g., face-grooming followed by body-grooming) represents a very well-known fixed action pattern (Kalueff et al. 2015). Similarly, in social dynamics among primates, a threat gesture carries a completely different weight if it follows a submissive posture rather than a neutral approach (Amici and Liebal 2022). In short, while the discrete method excels at answering “*how many*”, “*how much*” or “*how long*”, it fails to capture the syntax of behavior, leaving crucial questions like “*in what order*” or “*why*” largely unanswered.

2.4.2. Behavioral sequences, transition matrices and probabilistic shifts

LSA and transition matrices in general move a step beyond simple quantifications by introducing relational constraints. A consistent advantage of a transition matrix is its ability to map the “stochastic architecture” of behavior. Instead of looking at events in isolation, a transition matrix focuses on the probability that one state will lead to another, allowing for a more sophisticated understanding of behavioral structure. This is particularly useful in providing, from a probabilistic perspective, a “snapshot” of the entire observation; in addition, matrices are also very useful in identifying dominant sequences and behavioral “loops” that characterize specific experimental conditions or pathological states. For example, in a rodent exhibiting stereotypic behaviors, a transition matrix may be able to reveal a rigid “cycle” between two given components of the behavioral repertoire that a simple frequency or latency of a behavioral component alone would miss. By visualizing these transitions, it is possible to assess the predictability of a given sequence showing, for example, how the administration of a drug or a specific pharmacological treatment might restore a normal, or at least more flexible, behavioral repertoire. On the other hand, the drawback is that most transition matrices are “*memoryless*”. As above mentioned, indeed, they are

metaphorically no more than snapshots representing an observation time-window: their main advantage is, at the same time, their greatest weakness. They typically only account for the immediate predecessor of an event, effectively ignoring longer-term dependencies. If a behavior is influenced by an event that occurred three or four steps back in the sequence, a standard transition matrix will likely fail to detect this connection, offering a somewhat “*short-sighted*” view of the overall structure. For example, consider a complex predatory sequence: the decision to attack or to flee might not depend exclusively on the opponent’s last move, but on a cumulative series of signals exchanged over the previous time (Mobbs et al. 2015).

LSA might be imagined as a step beyond transition matrices, in that it trades the “*blindness*” of matrices for a perfectly focused but unmodifiable gaze. The primary advantage of LSA is its ability to move beyond the immediate “next step” (or, Lag 1) to explore delayed dependencies (Lag 2, 3, etc.) between behavioral events. Unlike simple transition matrices, LSA allows researchers to determine if a specific behavior significantly predicts another even if they are separated by intervening events in the sequence. For instance, examining associations between adult interactional strategies and infant gestures, an adult’s gesture might not trigger an immediate response of the infant, but it may significantly predict a “reaching gesture” two or three steps later, i.e., after the infant has appropriately processed the stimulus (Romano et al. 2025). LSA is indispensable for uncovering these non-adjacent contingencies that would be invisible to a stochastic transition matrix. However, a significant drawback of LSA is its rigidity regarding the distance between events. Since the researcher must pre-define the specific lags to be analyzed (e.g., looking specifically at what happens 1 or 2 steps later), it may miss significant relationships that occur at variable or irregular intervals, a gap that only more fluid pattern analysis can bridge.

2.4.3. Complex patterns and hidden temporal architectures

When the research aims shift toward uncovering the deep organization of behavior, time becomes an essential factor and T-pattern analysis becomes the indispensable choice. Its greatest strength is the ability to reveal complex temporal architectures that are completely invisible to the naked eye. This approach allows researchers to reveal if and how events are connected across time, even when separated by variable intervals. It provides a truly holistic view, transforming raw data into a map of meaningful, recurring relationships.

The challenges associated with this method, however, are significant. It requires a higher “entry cost” in terms of both computational expertise and specialized software. Furthermore, the data collection phase must be exceptionally rigorous because any inaccuracy in recording the onset and offset of events can significantly degrade the quality of the analysis. Consequently, the cost-benefit ratio must be carefully evaluated, as the depth of insight gained requires a much larger investment of time and resources compared to traditional descriptive methods.

On the basis of these premises, the following sections will provide a comprehensive exploration of T-pattern analysis. As a sophisticated pattern detection, this methodology offers a rigorous approach for identifying hidden, non-random temporal structures that characterize the behavioral

flow of living organisms, moving beyond the constraints of individual evaluations such as frequencies or durations, fixed lags and memoryless transitions.

3. Sequences and Patterns in Time

Since any sequence of events unfolds in time, one might be tempted to regard it as a temporal pattern. Such an interpretation would be misleading. This issue is closely linked to the precise meaning of the term “*pattern*”.

3.1. Behavioral patterns

In general terms, a pattern may be defined as a model, template, or regularity identified within a set of data, objects, or events. The concept has wide applicability across numerous disciplines. For instance, in informatics and/or computer science, the so-called “*design patterns*” represent established solutions to recurring problems encountered in software development, while “*pattern recognition*” refers to a software’s ability to identify regularities within data. In mathematics, “*pattern*” refers to a series of numbers that follow a specific rule; in computer graphics, the term “*pattern*” denotes the design or background of a web page, whereas in design and fashion, the term refers to the motif or print of a cloth. In architecture, one of the first to provide a modern definition of the concept of “*pattern*” was Christopher Alexander, an architect, design curator of several buildings worldwide, and author of many elegant studies. He stated: “*Each pattern is a three-part rule, which expresses a relation between a certain context, a problem, and a solution*” (Alexander et al. 1977; Alexander 1979).

Surprisingly, the Alexander’s elegant definition can be applied, with the appropriate caution, to behavioral sciences as well. Keeping in mind that behavior is the result of a complex interaction between innate and acquired responses (Lorenz 1981), triggered by external (Tinbergen 1951a) or internal (Tinbergen 1951b) causal factors, then *a behavioral pattern can be defined as the way of acting (or reacting) that a subject tends to repeat when found in the same (or similar) context and faced with the same (or similar) problem/situation*. A clarifying example may be useful. Imagine being on the sidewalk of a very busy street with the need to reach the opposite side. Obviously, the first action involves checking for vehicles from the right and left, followed by starting to move toward the opposite sidewalk. Implicit in this scenario is the requirement that the following events do unfold in a rigid sequential order: (1) approaching the street, (2) looking left and right, (3) confirming the absence of approaching vehicles from both directions, and (4) moving toward the opposite sidewalk. Notably, these four points will remain entirely unchanged in their *consecutio* every time we cross a street, that is, every time we find ourselves in the same situation. Deviating from this necessarily rigid order (for instance, starting to cross before evaluating the arrival of

vehicles) is not even a hypothesis to be considered, for obvious reasons. Yet, when speaking of behavior, such necessary rigidity in the sequence is not enough. It is essential to include *a factor just as important as the correct sequence of events itself: time and, more specifically, timing among events*.

The four moments of the example described above must necessarily occur not only in a rigid order but also with an equally rigid timing, the latter being at least *as important as the order of the events themselves*. This simple example does highlight the extreme importance of both correct sequencing and temporal links in shaping functional behavior that is appropriate to the context. The significance of time in the study of behavior was well discussed by Professor Eibl-Eibesfeldt, who emphasized that, since behavior is composed of patterns unfolding over time, any study intending to examine behavior must necessarily engage with sequences of events that are intrinsically not always easy to assess (Eibl-Eibesfeldt 1970).

3.2. The Temporal Context as Interpretive Frame

Figures 2 and 3 illustrate, albeit in markedly different ways, the behavior of rats evaluated in the Hole-board, during 10 minutes of observation. Beyond the obvious considerations regarding the evident representational differences, one aspect immediately stands out: many of the links highlighted in Fig. 2 are absent in Fig. 3. The fundamental difference lies in the fact that while Fig. 2, originating from the analysis of a transition matrix, provides a “snapshot” of the entire observation period, so offering a general overview of the transition probabilities, Fig. 3, originated from TPA, reveals the *actual* behavioral structure of the rodent by showing the real-time temporal constraints between the various elements of the animal’s repertoire. Well beyond merely representational aspects, it is therefore on a conceptual and theoretical level that the most remarkable differences exist. The weight of these differences is immense.

Methodologies that allow for the study of temporal patterns, such as TPA, are the most comprehensive because they represent the final integration of all preceding levels. Metaphorically speaking, if discrete phenomena are the bricks and sequences are the walls, then temporal patterns are the entire building. The reasons for this are multi-factorial.

- a) First and foremost, these analyses overcome the limitation of so-called “background noise”. Unlike simple sequences, the study of patterns can identify relationships between events even when time elapses between them or other irrelevant behaviors do occur. In other words, they do not lose information simply because it is not immediately successive: they manage to see the logical structure of behavior through apparent chaos.
- b) Secondly, they reveal the hierarchy of behavior. In this regard, it must be emphasized that behavior is not linear, as one might instead think when observing a sequence of behaviors written on a piece of paper or a spreadsheet. A complex pattern is often composed of simpler patterns, which in turn contain sequences of discrete acts. These analyses are the only ones that allow for the reconstruction of the entire hierarchical architecture, showing how micro-

movements (for instance, on the scale of milliseconds) aggregate into macro-behavioral strategies (on the scale of minutes or hours).

- c) Furthermore, methodologies for studying temporal patterns provide essential information on the distance between events; quite simply, while other approaches are limited to counting (i.e., how often does it happen?) or ordering (i.e., what happens next?), the study of patterns analyzes the temporal distance between acts. These are, therefore, analyses capable of revealing whether a relatively constant interval exists between two events. This information is crucial: a relatively invariable time interval suggests an underlying biological or cognitive mechanism (e.g., the brain's processing time) that a simple frequency would totally ignore.
- d) Finally, but no less importantly, thanks to these analytical methodologies, it is possible to reduce potential errors of interpretation, which are always behind the corner. Isolating a single act (discrete phenomenon) often leads to erroneous conclusions because the context is lost. Studying the temporal patterning means studying the events within their natural temporal context. Only temporal patterns provide the "code" to correctly interpret the meaning of behavior.

As mentioned in section 2.3., the approach that enables the evaluation of temporal relationships between events over time is known as T-patterns analysis (TPA) and utilizes a software tool called THEME (Patternvision LTD, Reykjavík, Iceland). The core of TPA is not merely the sequence of events itself, but the statistical calculation of their temporal proximity. In brief, if an event A is followed by an event B more frequently than would be expected by chance, and if this relationship occurs within a specific time interval $[t_1, t_2]$, a "critical interval" is identified. This approach is revolutionary because it allows the algorithm to filter out background noise. In other words, if 10 or 100 irrelevant events (D, E, X...) occur between event A and event B, the analysis ignores them and still detects the connection between A and B. Metaphorically, it is like listening to a conversation in a crowded room: the brain filters out background noise to focus on the speaker's voice, which is what truly matters. Once a pattern (A B) is identified, the algorithm treats it as a single unit and searches for whether this new simple pattern is temporally linked to another event C, producing ((A B) C). This search process follows a bottom-up progression, creating a "tree structure": at the base, we find individual behaviors; moving upward, we discover how these combine into more complex expressions, up to macro-strategies. Furthermore, T-patterns are by definition recursive structures that can be presented textually as strings or graphically as tree structures, both of which indicate their hierarchical nature (Magnusson 1996, 2000, 2004, 2020, 2023a).

All of this is extremely compelling as it suggests that human and animal behavior is not a continuous, amorphous flow, but is governed by a true grammar. *Meaning lies in the relationship: just as in any language where a letter holds no meaning in isolation but acquires it within a word and then a sentence, so it is with a motor act*: its meaning changes depending on when it occurs relative to others. To understand the depth of this concept, let us consider another simple example. Imagine studying the dynamics of romantic affinity between two distinct couples, Couple A and Couple B, during a dinner. In this context, we wish to pay particular attention to one of the most common responses in the human species: the smile. This signal conveys relaxation and well-being to the partner: a powerful tool for assessing affinity. In Couple A, the partners smile numerous times following funny episodes shared with one another. In Couple B, conversely, only a few smiles are recorded. Thus, one might easily conclude that there is greater affinity in Couple A, where smiles are far more frequent. However, a closer analysis reveals that in Couple B, the smiles, though few,

always do occur within a specific temporal distance from a preceding brief mutual glance. In which of the two couples are we now more inclined to believe a deeper affinity exists? The answer is self-evident. In the second couple, *the meaning does not reside in the smile itself, but in the temporal vector that links it to the preceding gaze.*

This example, trivial in its simplicity, effectively illustrates how misleading a purely quantitative evaluation of an event can be. Conversely, it highlights how essential it is to examine the temporal relationships between events that appear distant but are strictly and indissolubly linked by a causality. This causality is inherently elusive, as it may be shared by two events that are not immediately consecutive. Returning to the aforementioned example, TPA demonstrates that the frequency of smiles is far less significant than their temporal positioning within the flow of interaction. This leads to a fascinating hypothesis: *from a structural standpoint, the essential unit of analysis is not the actions produced by the subject, but rather the time interval that connects them. It is within that interval between the glance and the smile that the meaning of communication, behavior, and their underlying grammar resides. Without these fundamental yet invisible temporal constraints, behavior would ultimately be stripped of the greater part of its meaning.*

3.3. From Theory to Empirical Evidence

Here, we have focused on the conceptual aspects regarding the importance of temporal analysis, specifically TPA, while simultaneously emphasizing the value of analyses concerning “lower levels”, such as discrete events or sequences. TPA is built upon a solid theoretical and conceptual foundations. These have been extensively discussed over the last three decades in various papers by its creator, Prof. Magnus S. Magnusson ([Magnusson 1996, 2000, 2004, 2020, 2023a](#)), which detail the theoretical underpinnings of TPA. Furthermore, several monographs, reviews, and books on the subject have been produced ([Magnusson et al. 2016, 2023b](#); [Anguera et al. 2023](#); [Casarrubea et al. 2015](#); [Casarrubea and Di Giovanni 2020](#)), enriching the knowledge base for those wishing to further investigate this elegant methodology.

Beyond the aforementioned works, TPA has demonstrated remarkable versatility across diverse fields, offering novel perspectives on the organization of behavior in both human and non-human subjects. Over the last thirty years, a wealth of evidence has systematically highlighted the advantages of TPA compared to more conventional quantitative approaches. Specifically, following the formulation of T-pattern theory, which enabled the detection of “hidden” spatio-temporal configurations in intra- and inter-individual interactions ([Magnusson 1996, 2000](#)), this methodology has been applied to a broad spectrum of research areas. These include the study of human-animal ([Kerepesi et al. 2005](#)) and human-artificial agent interactions ([Kerepesi et al. 2006](#)), the investigation of neuropsychiatric pathologies such as schizophrenia ([Lyon et al. 2004](#); [Kemp et al. 2008](#); [Kemp et al. 2016](#); [Sandman et al. 2012](#)), the analysis of movement disorders ([Aiello et al. 2020](#)), hormonal correlations ([Hirschenhauser et al 2002](#)). T-patterns have also proven prolific in preclinical research on primates ([Gunst et al. 2020](#); [Cenni et al. 2020, 2021](#)) and rodents where it has been successfully employed in models of Tourette syndrome ([Santangelo et al. 2018](#)) and Parkinson’s disease

(Casarrubea et al. 2019a), as well as in the study of stereotypes (Bonasera et al. 2008), exploratory response in tests such as the Elevated Plus Maze (Casarrubea et al. 2013, 2014) and the Hole-Board (Casarrubea et al. 2010a, 2023, 2024), and the study of feeding behavior (Casarrubea et al. 2019b).

Viewed in this light, the T-patterns detected in behavior appear to belong to a wider family of self-similar, hierarchically organized temporal structures observable across biological scales, from molecular and neuronal events to dyadic interaction and collective life. The same kind of statistically constrained temporal relations that bind a glance to a smile in a couple at dinner also recur, in different guises, in the timing of cellular signalling, in the syntax of birdsong, and in the rhythms of social systems (Magnusson 2020, 2023a). The temporal architecture revealed by TPA is therefore not merely a methodological convenience but a candidate organizing principle of living systems - a behavioral grammar whose alphabet is time itself.

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CRedit authorship contribution statement

Magnus S. Magnusson: Writing - original draft, conceptualization; **Maurizio Casarrubea:** Writing - original draft; **Stefania Aiello:** Writing - review & editing; **Teresa Pasqua:** Writing - review & editing; **Giuseppe Crescimanno:** Writing - review & editing; **Giuseppe Di Giovanni:** Writing - review & editing

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<<<Captions of figures and tables after References section>>>

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Captions of figures and tables

Fig. 1 - In behavioral analysis it is possible to distinguish between isolated events, sequences, and patterns.

Fig. 2 - Path diagram representing transition probabilities among behavioral patterns observed in a group of rats tested in the Hole-Board. Thick arrows = probability > 0.25; thin dashed arrows = probability < 0.25. Edge-Sniff (ES): rat sniffs the border of one of the four holes; Head-Dip (HD): rat puts its head into one of the four holes; Immobile-Sniffing (IS): rat sniffs the environment standing on the ground; Climbing (Cl): rat maintains an erect posture leaning against the Plexiglas wall; Walking (Wa): rat walks around sniffing the environment. Modified with permission from (Casarrubea et al 2009).

Fig. 3 - T-pattern consisting of five behavioral events (a) occurring in 15 subjects (b). An increased detail on subject #2 (c) illustrates onsets and occurrences of the T-pattern during 10 min observational window. For abbreviations see figure 2. Modified with permission from (Casarrubea et al 2010b).

Tab. 1 - Tools and approaches employed in the study of isolated events, sequences, and patterns.

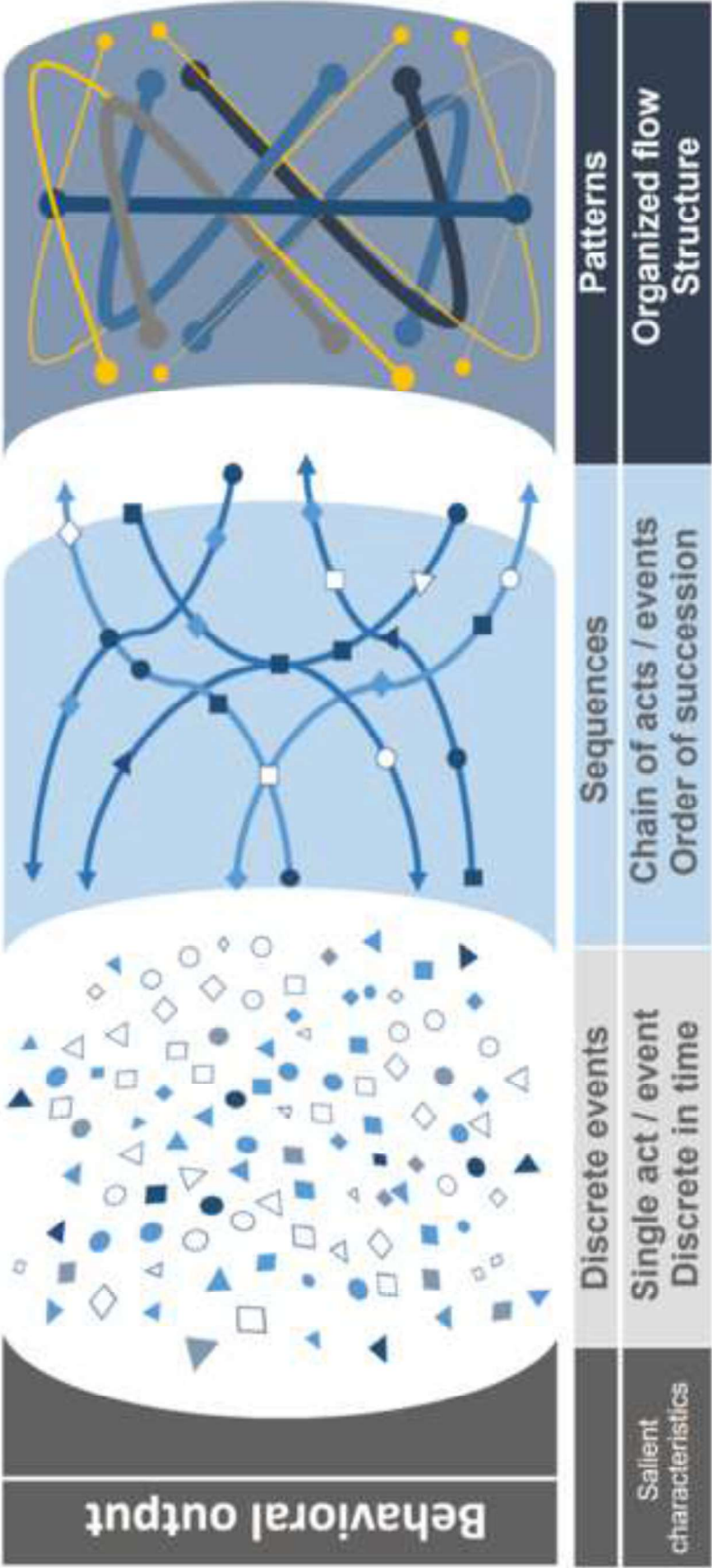
Highlights

- Time is not a mere background condition but a core component essential for organizing, expressing, and interpreting the actions of living beings.
- The paper evaluates specialized tools and approaches, including software coders, transition matrices, and T-pattern analysis, to study the relationships among behavioral components
- Emphasis is placed on how temporal relationships and structural regularities define the intrinsic architecture of action and its underlying meanings.

	Instrument/Approach	Field of Application	Software or Hardware tool
(A) Discrete events	Behavioral Coders, Keyloggers, Button Boxes and Response Pads	Annotation and study of motor acts and single events (e.g., a scratch, a jump, a lick, etc.)	The Observer (Noldus et al 2000 ; Noldus IT 2026a); Boris (Friard and Gamba 2016); Solomon Coder (Péter 2017); JWatcher (Blumstein and Daniel 2007); Lince Plus (Soto-Fernández et al. 2022); Psychtoolbox, PsychoPy (Bridges et al 2020)
	Automatic detection systems	Passage events, contact events, movements	Infrared Sensors and switches, e.g., utilized in Operant Chambers (Soares et al 2016); Movement sensors and accelerometers, e.g. utilized in actigraphy (Morgenthaler et al. 2007 ; Keenan et al 2024 ; Zampogna et al 2020)
	Video tracking systems, Eye-Trackers, Face Readers	Automatic annotation of movements; annotation of movements where millisecond-order evaluations are essential	Wearable eye trackers (Kassner et al 2014); screen-based eye trackers (Holmqvist 2017); Ethovision (Noldus et al 2001 ; Noldus IT 2026b); FaceReader (Noldus 2026c)
(B) Sequences	Lag Sequential Analysis (LSA)	Predictivity (e.g. does act A predict act B?)	GSEQ (Bakeman and Quera 2011)
	Transition matrices, Markov Chains, adjusted residuals	Probability of transition (e.g. what is the transition probability between states?); transitions occurring significantly more or less often than expected	R (package "markovchain") (R Core Team 2026); MatMan (de Vries et al 1993)
	Network Analysis	Evaluation of central behaviors in the sequence	Gephi (Bastian et al 2009); Cytoscape (Shannon et al 2003)

(C) Patterns	Pose Estimation and Machine Learning	Detection of Motor Patterns	DeepLabCut (Mathis et al 2018); SLEAP (Pereira et al 2022); B-SoiD (Hsu and Yttri 2021)
	State-Space Grids	Trajectories and attractor states	Gridware (Hollenstain 2013)
	T-pattern analysis (TPA)	Temporal relationships between events over time	Theme (Magnusson 1996, 2000, 2020 ; Magnusson et al 2016)

Figure 1 - Events, Sequences & Patterns



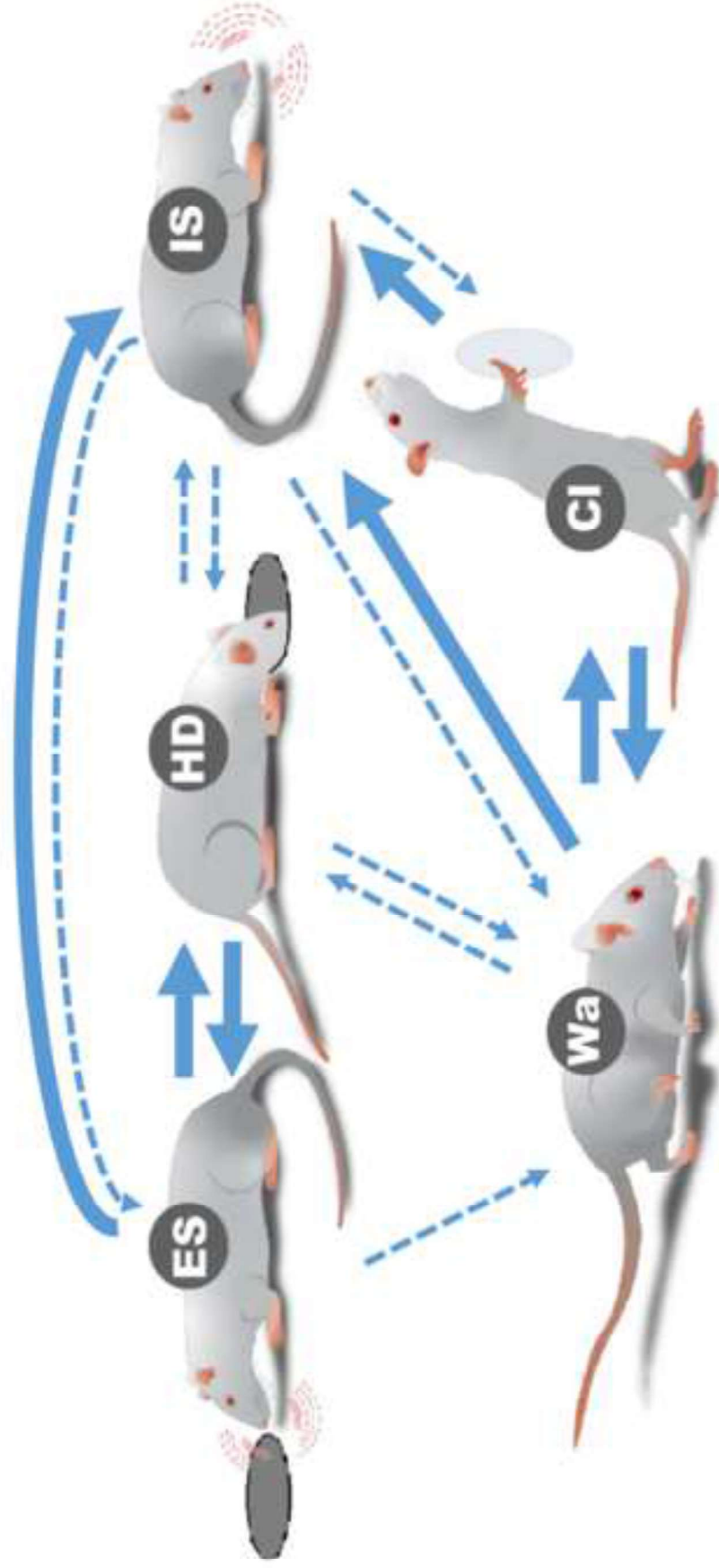
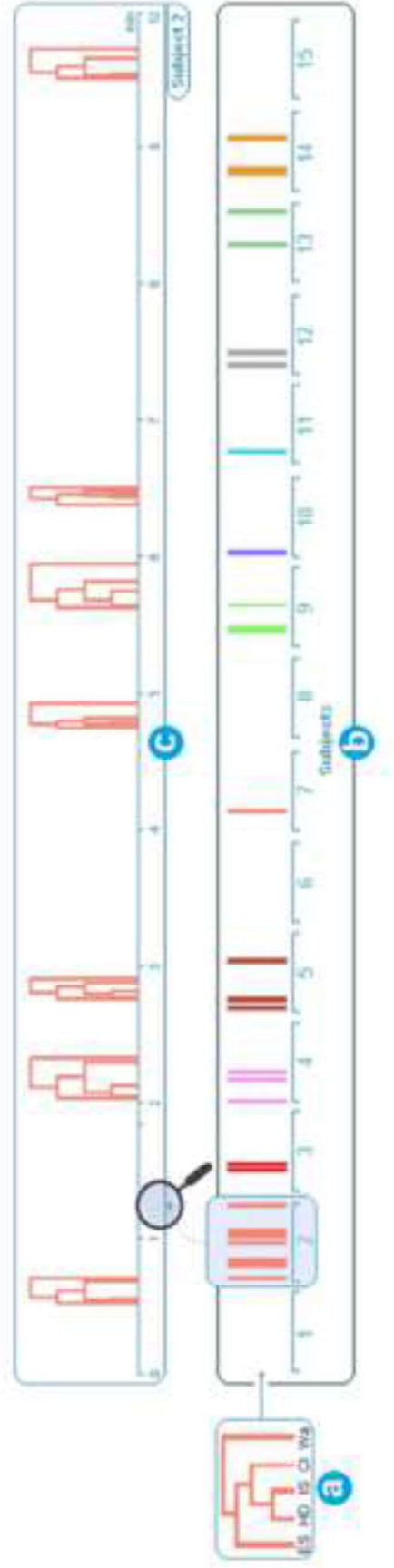


Figure 3 - Example of T-pattern



Declaration of competing interest

The authors declare no conflicts of interest, financial or otherwise, regarding the publication of this work.