Application of a Network-Analysis Algorithm for the Definition of Locations in Open Field Behavior: How Rats Establish Behavioral Symmetry in Spatial Asymmetry

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Abstract

The present study applied network analysis to scrutinize spatial behavior of rats tested with symmetrical or asymmetrical layout of 4, 8, or 12 objects along the perimeter of a dark, round arena. We considered locations as the units of a network (nodes), and passes between locations as the connections of the network. In terms of Ethovision parameters, there were only minor differences between rats tested in either symmetrical or asymmetrical object layouts. However, network analysis revealed substantial difference in the behavior between the layouts. For the network analysis, we first defined locations in the environment, where each 'location' was a cluster of stopping coordinates (defined as no progression for at least 1 second) extracted from Ethovision. From the set of locations and the passes between them we extracted the network analysis parameters: for each node-degree,clustering coeficient and shortest mean path were calculate. In addition the average network degree,clustering coeficient and shortest mean path were extracted for each rat. It was found that behavior in either a symmetrical or asymmetrical layout of 4 objects, the key locations coincided with the objects. However, in the asymmetrical layout with 4 objects, additional key locations were spaced at a distance that was identical to the distance between other objects, forming behavioral symmetry among the key locations. In other words, it was as if the rats imposed behavioral symmetry in their spatial behavior in the asymmetrical environment. We suggest that wayfinding was easier in symmetrical environments, and therefore, when the physical attributes of the environment were not symmetrical, rats behaviorally established a symmetric layout of key locations, thereby gaining a more legible representation of the environment despite its more complex physical structure. Altogether, the present study adds a behavioral definition for a location, a term that so far has been mostly discussed according to its physical attributes or neurobiological correlates (place, border and grid neurons). Moreover, the network analysis enabled to identify a location and to assess its importance, even when that location did not have distinctive physical properties.

Materials and Methods

Ethical statement

This study was carried-out under permit # L-10-013 of the IACUC of Tel-Aviv University. For the purpose of this study, male Wistar rats (n = 16; age 3 months; weight 250–300 g) were housed in a temperature-controlled room (21°C) with 12/12 h light/dark cycle (dark phase 8:00 to 20:00). Rats were held in standard rodent cages (40 X 25 X 20 cm; 2 rats per cage) with sawdust bedding and were provided with free access to water and standard rodent chow. Each rat was marked with a waterproof marker on its tail, and accustomed to handling - 10 minutes a day for one week. Rats were maintained and treated according to the institutional guidelines for animal care and use in research.

Apparatus

Rats were tested in a round arena, 200 cm in diameter, surrounded by a 50 cm high tin wall. The arena was placed in a temperature-controlled (21 ±1 °C) and light-proofed room. The arena floor was covered with a navyblue PVC layer. During testing, the room was completely dark, illuminated only by infra-red light invisible to rats (Tracksys, IR LED Illuminator; UK, with a 830 nm wavelength filter). Trials were recorded by a video camera (Ikegami B/W ICD-47E, Japan) placed 2.5 m above the center of the arena, providing a top view of the entire arena. Footage was saved on a DVD device (Sony RDR-HXD 870, Japan). Each rat underwent three trials with 4, 8, and 12 objects respectively, in only one of the following two object layouts. Objects (black cement blocks; 6.5 X 6 X 6 cm), were placed in either a symmetrical or an asymmetrical layout along the arena walls. In the asymmetrical layout, distance between objects was established randomly, with at least 25 cm between objects, preventing the rats from touching two objects at the same time. Overall distance between objects was equal on average for both layouts in each trial.

Procedure

Sixteen rats were randomly assigned to two groups of eight rats, each undergoing three trials in an arena with either a symmetrical or an asymmetrical layout of objects. Each rat was individually tested on alternate days with an increasing number of objects, starting with 4, then 8, and finally 12 objects. We did not include the counterbalanced paradigm (18, 8 and then 4 objects) since our past studies indicate that this procedure did not affect the results [1-2]. At the beginning of each trial, a rat was placed at a fixed start location next to the arena wall, and its behavior was recorded for 20 minutes. The arena was wiped with detergent between successive trials. All testing took place during the dark phase when rats were most active.

Data acquisition and analysis

For path analysis, the arena was divided into the following virtual areas:

Perimeter - a 15 cm strip along the arena wall.

Center - the remaining central area of the arena (excluding the perimeter area).

Object area - a 25 X 25 cm square, around each object. Since objects were placed along the perimeter, object areas were of course located within the perimeter area.

The paths of movement of the rats in these areas were tracked from the video files using ‘Ethovision XT 7’ (Noldus Information Technologies, NL), a software that provided the coordinates of the center of mass of the rat five times per second. The following parameters were extracted for further analysis with 'Microsoft Excel 2007':

Distance traveled - the cumulative metric distance (m) traveled over 20 minutes.

Duration - the time spent (min) at each of the arena areas.

Travel between center and perimeter - incidence of crossing between center and perimeter areas.

Duration at an object - the time spent (min) in an object area.

Visits to an object - the number of entries to an object area.

Network analysis

For network analysis, behavior was considered as a set of locations and the transitions between these locations. In this representation, we defined local parameters that referred to the behavior within a specific location, and global parameters that referred to the behavior in the entire arena. Custom-designed software ('Huldot' by Michael Liberthal) was used to identify locations of interest for the rats, as reflected by their X-Y stopping coordinates inside the arena. We defined a stop as no progression for at least 1 second. ‘Huldot’ was based on an algorithm that was based on the stopping behavior [3-4], its mathematical principles much resemble the City Clustering Algorithm (CCA) [5-6], yet it represents a novel approach to the study of spatial behavior. Using the ‘Huldot’ algorithm, we identified the rat’s first stopping coordinate and defined it as node1. We then added to node1 all the stopping coordinates that were located at $d \leq l$ from the first stop (where d represents the measured

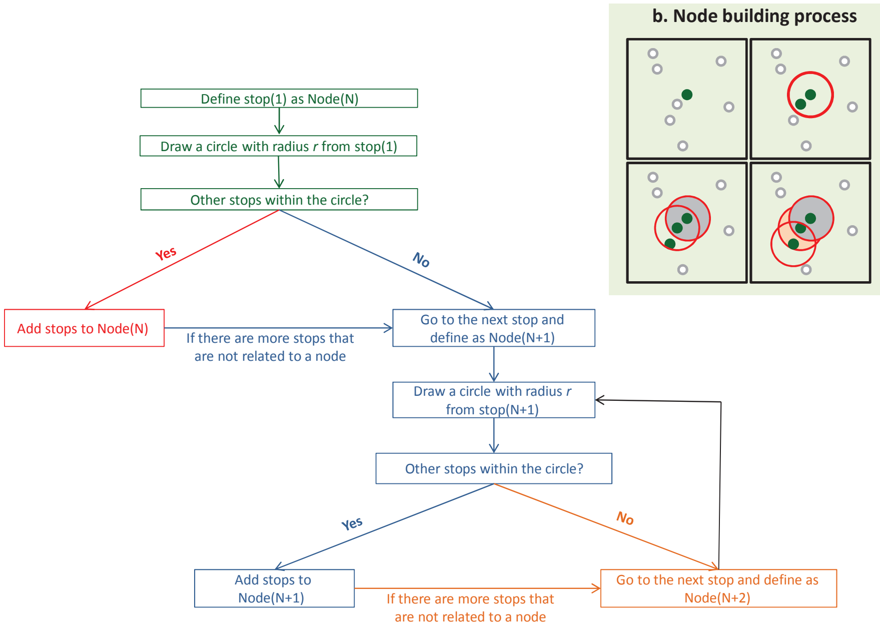

Figure 1. The algorithm for transforming stopping coordinates into a network node (**a**) and a visualized process of building a single node (**b**). (taken from Weiss et at. PlosOne 2012, under revision).

distance between the stops and l represents a "unifying criterion" that was set to 12 cm – about half a rat's body-length). We continued adding new stops to node1 until there were no more stops at $d \leq l$ from any of the stops included in node1. We then identified the rat's next stop (not included in node1), defined it as node2, and repeated the process (see Figure 1).

The distance between stopping coordinates was calculated, and stopping coordinates within a 12-cm diameter were assigned to the same node. This diameter was found to be the best fit according to the following considerations: physically, this diameter (12 cm) had to be less than 14.5 cm (which is half the shortest distance between objects), as otherwise stopping coordinates at two adjacent objects could be attributed to one node at an intermediate distance between the two objects (see Figure 2). The 12-cm diameter also had to be greater than 9 cm in order to prevent the splitting of stopping coordinates at the same object into two separate nodes (see Figure 2). Within this diameter range, the 12-cm diameter was the best fit for all animals. Altogether, the algorithm provided a method by which to define locations in the spatial behavior of the rats, offering a useful definition irrespective of network analysis. In the present study, however, this definition of locations (nodes) was a prerequisite for network analysis, enabling us to view the behavior as a network comprised of nodes (node = clusters of stopping locations), and of links between these nodes (link = pass from one node to another). Moreover, once the nodes and the links between them were established, it was possible to refer only to the topology of the behavior while ignoring the metric distance between actual stopping coordinates, and to analyze the behavior only in terms of the nodes and links that constituted the network (see Figure 2e).

Stopping coordinates: - these are as the x-y coordinates of a single rat, as extracted from the tracking system (Ethovision). The large black circle represents the arena perimeter, each red dot represents a stopping coordinate at which the rat stopped for one second or longer, and the black squares represent the location of the objects.

Nodes under the application of a 12-cm circle around the additional stopping coordinate:- As shown, with this diameter the nodes (circles) coincide with the objects and behavior.

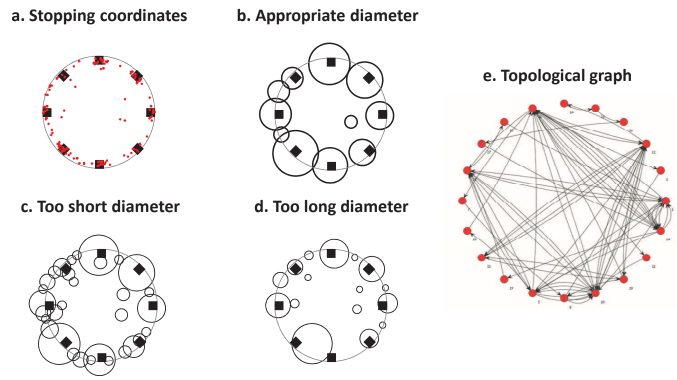

Figure 2. The rationale for establishing the criterion of 12 cm diameter and the transformation of stopping coordinates into a network is illustrated for one rat. (taken from Weiss et at. PlosOne 2012, under revision).

Nodes under the application of a 9-cm circle around the additional stopping coordinate:- As shown, with this diameter stopping coordinates around the same object split into several nodes, resulting in a mismatch between behavior and nodes.

Nodes under the application of a 14-cm circle around the additional stopping coordinate:- As shown, with this diameter the bottom node encompasses the stopping coordinates of two objects (see the red dots of these objects in a.).

Topological graph:- The presentation of the network after the transformation of stopping coordinates into nodes (red circles). Arrows between nodes represent the links (passes) between nodes. Note that the location of a red circle does not represent the physical location of that node. Likewise, the circles that represent the nodes in b-d do not represent the real size of the node but the number of stopping coordinates included in that node.

Local network parameters

Once nodes and links had been defined, the following parameters were provided by 'Huldot' for each node in the network:

Degree/Connectivity (**k**) - the number of links that a node has with other nodes (i.e. the number of neighbors that a node has).

Clustering coefficient (**C**) - the number of actual links between the neighbors of a specific node, divided by the total number of possible links that could occur between them (“how many of a node’s neighbors are also each other’s neighbors”). Clustering coefficient (C) is a value between 0 and 1, representing the level of connections between all of a node's neighbors (what portion of a node's neighbors are themselves neighbors). This was calculated as follows: $\boldsymbol{C} = \frac{2|e_{jk}|}{k_i(k_i-1)}$ where $\boldsymbol{e_{jk}}$ is the number of links between node $\boldsymbol{i}$ to other neighbors, and $\boldsymbol{k_i}$ is the number of node i neighbors.

Shortest path length (**l**) - the minimal number of nodes needed to be traveled in order to reach all the nodes in the network from a specific node.

The above parameters shed light on three different aspects of the nodes, indicating the relative importance of the nodes within the network. For example, a node with a high degree (**k**) and a low shortest path length (**l**) would be typical to a key node (hub) for travel within the network.

Global network parameters

While the above parameters refer to specific nodes, additional parameters were calculated for the entire network of each rat, as follows:

Total number of stops

Total number of nodes

Average network degree (**<k>**) - the average number of links per node (the average of the above degree values of all nodes). This parameter represents the connectivity of the network.

Average network clustering coefficient (**<C>**) - the average of the above local clustering coefficients of all nodes.

Average network shortest path length (**<l>**) - the average of the above shortest path lengths of all nodes. This parameter reflects the minimal number of nodes that needed to be traveled in order to reach from any node to any other node in the network.

Network density (**d**) - the ratio of the number of actual links divided by the number of theoretically possible links between all nodes.

"Key nodes" - nodes that encompass 10 or more stopping coordinates were defined as key nodes. The value of 10 stopping coordinates was set as a threshold based on ranking all nodes according to the number of stopping coordinates clustered within them. A two-fold difference from other nodes was noted in the layout with highest ranking nodes, which were thus separated and defined as key nodes with 10 as their minimal number of stopping coordinates.

Statistics

Data were compared by either a two-way ANOVA with repeated measures followed by a Tukey post hoc test, or by means of a Student's *t*-test. Alpha level was set to 0.05.

References

1. Eilam, D. (2003), Open-field behavior withstands drastic changes in arena size. *Behavioural Brain Research* **142**(1-2), 53-62.

2. Ben-Yehoshua, D., Yaski, O., Eilam, D. (2011). Global movement in a changing environment: spatial behavior in rats is shaped by global and not by local geometry. *Animal Cognition* **14** , 341-350.

3. Eilam, D., Golani, I. (1989).Home base behavior of rats (Rattus norvegicus) exploring a novel environment. *Behavioural Brain Research* **34**(3), 199-211.

4. Golani, I., Benjamini, Y., Eilam, D. (1993). Stopping behavior: constrains on exploration in rats (Rattus norvegicus). *Behavioural Brain Research* **53**, 21-33.

5. Rozenfeld, H.D., Makse, H.A. (2009). Fractality and the percolation transition in complex networks. *Chemical Engineering Science* **64**(22), 4572-4575.

6. Rozenfeld, H.D., et al. (2008). Laws of population growth. Proceedings of the National Academy of Sciences of the United States of America **105**(48) 18702-18707.

7. Weiss, S., Yaski, O., Eilam, D., Portugali, J., Blumenfeld-Lieberthal, E. (2012) Network analysis of rat spatial cognition: behaviorally establishing symmetry in a physically asymmetrical environment. PlosOne - under revision.