Effect of the Ecological Location of a Water Source on Entropy and other Spatio-temporal Behavioral Features: An extended and systematic replication

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Abstract

The continuous analysis of spatial behavioral-dynamics under stimuli-schedules has been a scarcely studied field in experimental psychology. A recent study conducted in our laboratory suggest that the features embedded in the spatial dynamics of behavior are affected by stimulus-schedules, at least, as much as features embedded in discrete responses. In that study we compared the spatial behavioral dynamics under two time-based schedules (fixed vs variable time) of water delivery, and two different locations of water delivery (delivery in central zone vs. perimetral zone) on a Modified Open Field System (MOFS). The present work replicates those findings taking in consideration previously uncontrolled variables. In Experiment 1, three subjects were exposed to a Fixed Time 30s water-delivery schedule. In the first phase the water dispenser was located at the perimetral zone. In the second condition, the water dispenser was located at the central zone. Each location was presented for 20 sessions. In Experiment 2, conditions were the same, but a Variable Time schedule was used. Measures of entropy were used to describe the spatial behavioral dynamics. We found higher levels of entropy under central location of water delivery than in the perimetral location; and higher entropy under Fixed than Variable Time Schedule, confirming previous findings but under different sequences of dispenser locations. In general, a well-differentiated dynamic between experimental conditions was observed in terms of direction (distance to the dispenser) and variation (entropy) of spatial behavior. These findings are discussed under a systemic, parametric, ecological, and non-mediational framework.

Keywords: Spatial dynamics; time-based schedules; recurrence; entropy; rats; animal behavior.

In recent studies, the relevance of the integration of the ecological approach with the arbitrary approach in behavioral sciences has been acknowledge (Cabrera et al. 2019; León et al. 2020; Timberlake, 1990). While the ecological approach emphasizes the study of behavior in a context in which stimuli and responses have ecological relevance (e.g., water seeking behavior, exploratory behavior, etc.) and the role of displacement activity on changes in the contact stimulation, the arbitrary approach is characterized for its emphasis on the systematic variations of temporal parameters of stimuli and its effect on the rate of an arbitrary and discreate response.

An integrated system of discrete responses (e.g., lever pressing, nose poking, head inputs) and displacement patterns (e.g., displacement routes) could bring the arbitrary approach closer to the ecological approach since it allows, from the arbitrary approach, to characterize and analyze behavioral patterns with ecological relevance (e.g., water-seeking behavior) but in a parametric perspective.

Focusing on Spatio-temporal variation in displacement patterns, studies in behavioral science have shown that, using non contingent schedules, changes in Spatio-temporal variation in displacement patterns can be modulated by the schedule of reinforcement (Eldridge et al., 1988; Van Hest et al., 1986), the number of available dispensers in the experimental chamber (León et al. 2020) and variations in the location of reinforcement (León et al. 2020), among others variables.

For example, from a parametrical approach, in a study conducted by León et al. (2020) the authors analyzed changes in displacement patterns under different combinations of FT and VT schedules with water delivered in a constant or varied location. They found more variable patterns in the combination of VT in a varied location than with FT in a single location.

On the other hand, studies from the ecological perspective (Martinez & Morato, 2004; Yaski. et al. 2011; Whishaw et al. 2006), using the open field paradigm have found

differences in spatio-temporal patterns of behavior regarding different zones of the experimental chamber, even without programed contingencies in it. For example, Yaski and colleagues found that rats exposed to an open field arena concentrated their displacement patterns in the peripheral zones in comparison to the central zones, they also found a higher velocity in the rat's movement in the central zone in comparison to the peripheral zone. For this authors different zones of the open field arena have different ecological relevance, so the close zones serves as a refuge or "safe area" while the central zone is an "insecure area" for organisms.

In a more recent study León et al. (2020) conducted a study bringing together the ecological and the parametrical approach. Considering the two previously mentioned findings, they compared the spatial behavioral dynamics under two time-based schedules Fixed Time (FT, Experiment 1) and Variable Time (VT, Experiment 2) of water delivery on two different locations: perimetral zone and central zone on a Modified Open Field System (MOFS). Subjects in each experiment were 3 experimentally naïve, water deprived Wistar-rats. In Experiment 1, water was delivered according to a FT 30-s schedule. In Condition 1, water was delivered at the Center of the experimental chamber; in Condition II, water was delivered close to a wall of the chamber. Each condition lasted 20 sessions, each session lasted 20 minutes.

For both schedules, FT and VT, with the dispenser locater at the center of the chamber, the distance from the rat to the dispenser was higher than the distance to dispenser when it was located close to the wall. The accumulated time of stays in different zones of the experimental chamber when the dispenser was located at the center, concentrated in the center and less in the peripherical zones, while with the dispenser located at the walls the distance was markedly lower. Also, the entropy index (an index that represents variations in displacement patterns) was higher when the dispenser was at the center than when it was located close to the wall. The difference among the VT and the FT schedules is that the under first one there was a lower behavioral dynamics in terms of variation of displacement patterns.

In Leon et al. study, the sequence of exposition to each location of the dispenser was the same for all subjects in both experiments, this is, center – wall, so it could be possible that the decrement in the dynamic of displacement patterns was due to a sequencing effect,

since it is well known that the behavior variation decreases with exposure to the stimulus-schedule through the sessions (Iversen, 2017). In order to determine if this was the case, it would be important to conduct a study in which subjects were presented with the location of the dispenser at the wall first and then at the center. If the previously mentioned results maintain, this would provide more robust evidence of the effect of the water location in the experimental chamber and its ecological relevance. On the other hand, if it were found results that differ from the ones reported by León al colleagues, it would mean that it was the effect of the sequence of exposition and that the water location is not a relevant factor in behavioral dynamics, as it was supposed to be.

In behavioral science, as well as in the general sciences an important component of the scientific practice is the reproducibility of the obtained results. A recent survey conducted in Nature (Baker, 2016) with 1576 researchers showed that more than 70% of them have tried and failed to reproduce the work of other scientists and more than 50% have failed to reproduce their own studies. The importance of the replication of findings lies down in that, as Sidman (1960) stated "The soundest empirical test of the reliability of data is provided by replication."

Considering the importance of replicating León et al (2020) results, data analysis and methodology, the purpose of the present study was to conduct a direct replication of León et al. study, presenting the location of water delivery in a reversed order: first with the water located at the wall and then at the center. In Experiment 1 a Fixed Time schedule was used while in Experiment 2 a Variable Time schedule was employed.

Experiment 1
Method

Subjects

Four experimentally-naïve, one male and three female, Wistar rats were used. All rats were three and a half months old at the beginning of the experiment. They were housed individually under a schedule of 23 hours of water deprivation with free access to water for 1 hour at the end of the experimental sessions. Food was freely available in their home cages. One session was conducted daily, 7 days a week. All procedures were conducted in agreement with university regulations of animal use and care and followed the official

Mexican norm NOM-062-ZOO-1999 for Technical Specification for Production, Use and Care of Laboratory Animals. One subject died of unknown reasons before finishing the experiment, because of this, the data for only three rats is reported.

Apparatus

A Modified Open Field System (MOFS) was used (León, et. al., 2020). Figure 1 shows a diagram of the apparatus. Dimensions of the chamber were 100 cm x 100 cm. All four walls of the chamber as well as the floor were made of black Plexiglas panels. The floor contained 100 holes of 0.8 cm located .95 cm from each other. A water dispenser, based in a servo system, made by Walden Modular Equipment was located close to the wall (Condition I) or close to the center of the MOFS (Condition II). When activated, it delivered 0.1 cc of water on a water-cup that protruded 0.8 cm from the floor of the MOFS in one of the holes. The MOFS was illuminated by two low-intensity lights (3 watts) located above the chamber and in opposite sides of the room to avoid shadow zones. Once delivered, water remained available 3 s for its consumption. A texturized black patch, 9x9 cm with 16 dots/cm, printed in a 3d printer was locate in proximity (5.5 cm) to the water dispenser to facilitate its location.

The experimental chamber was located on an isolated room on top of a table of 45 cm of height. The room served to isolate external noise. All programmed events were scheduled and recorded using Walden 1.0 software. Rats' movement was recorded by a Logitech C920 web camera, located at the center, 1.80 mts. above the experimental chamber. Tracking data was analyzed using Walden 1.0 software. This software recorded rats' location every 0.2 s in the experimental space using a system of x, y coordinate. The system recorded the rats according to their center of mass. Data files obtained from this software were then analyzed using MOTUS® and SPATIUM software.

Procedure

Subjects were exposed to two consecutive conditions in the same order (see Table 1). On each condition water was delivered using a Fixed Time (FT) 30 s schedule. When delivered, water remained available for 3 s. In Condition I, the water dispenser was located on the floor next to a wall of the experimental chamber (see Figure 1). In Condition II the water dispenser was located on the floor at the center of the experimental arena. Each

condition lasted 20 sessions. Each session lasted 20 minutes. Rats were directly exposed to the conditions without any previous training. The MOFS was cleaned using isopropyl alcohol between each experimental session.

Figure 1.
Representation of a Modified Open Field System
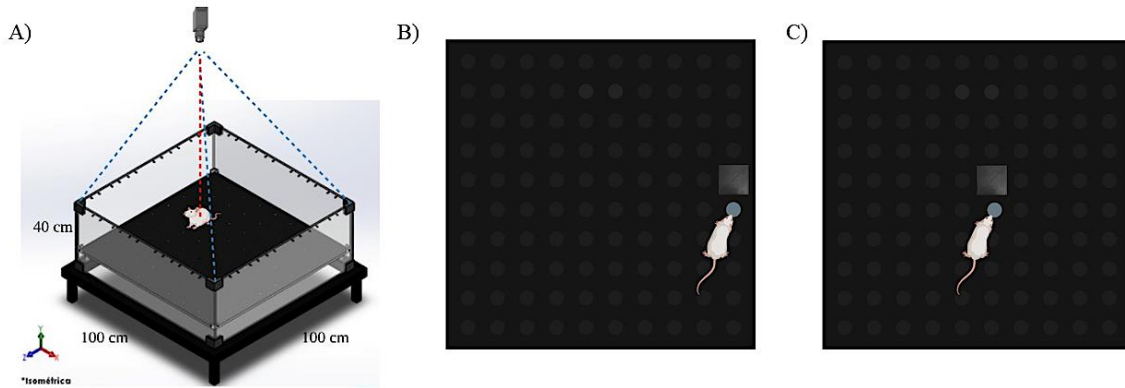
Note. The panel A shows an isometric view of the systems. The panel B represents the first condition with the dispenser on the wall-location, and the panel C represents the second condition with the dispenser on the center-location. The blue circle indicates the water dispenser location and the black square represents the texturized black patch. (Created with BioRender.com)

Table 1.
Experimental design of the Experiment 1 and 2

| Experiment	Water-delivery schedule	Condition I	Condition II
1	Fixed Time 30 s (FT) N=3	Wall	Center
2	Variable Time 30 s (VT) N=4	Wall	Center

Results

Figure 2 (left side) shows rats displacement in the experimental chamber every 0.2 s for a complete session for the FT schedule. The first column depicts routes for the last session of Condition I (wall) and the second column depicts routes for the last session of Condition II (center). The location of the rat in the first 0.2 s of water delivery is

Figure 2.

Complete routes for the last session for each rat for Condition I and II for Experiment 1(left panel) and 2 (right panel)
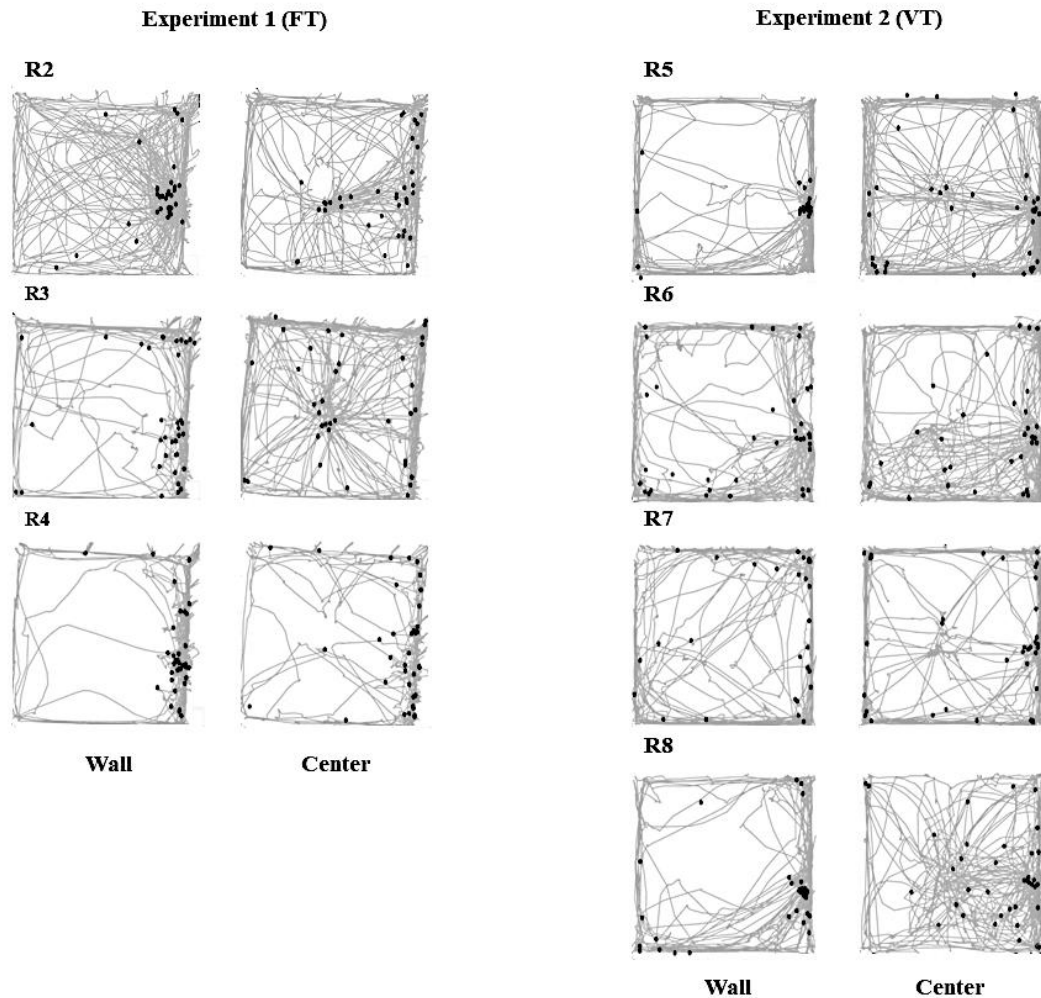
Note. Each panel shows the analogic routes in the MOFS for a complete session. Black points show rats' location in the arena at the first moment of water delivery (the first frame of 0.2 s of the 3 s of water availability). Each row depicts data for one rat, and each column depicts data for sessions 20 (last session of Wall-Condition) and 40 (last sessions of Center-Condition) for Experiment 1 (FT schedule) and Experiment 2 (VT schedule).

highlighted with a black mark. Since the water dispenser remained activated for 3 s, it was still possible that rats contacted the drop of water after the first 0.2s so the marks may not exactly corresponds with the number of drops of water consumed. In Condition I, for the three rats, the patterns of displacement were located predominantly at the walls of the chamber, although there were some crossings between walls. In that condition, rats location

at the time of delivery was close to the dispenser. In condition II, for all rats, a back and forth pattern between the walls and center of the chamber was found, rats location at the time of delivery for R2 and R3 was distributed between the wall and the center of the chamber (close to the dispenser). Location of R4 at time of delivery was away from the dispenser for most of the session.

Figure 3.
Normalized value of the distance from the rat to the dispenser every 0.2 s (gray dots) for the last session for each rat for Condition I and II for Experiment 1(left panel) and 2 (right panel)
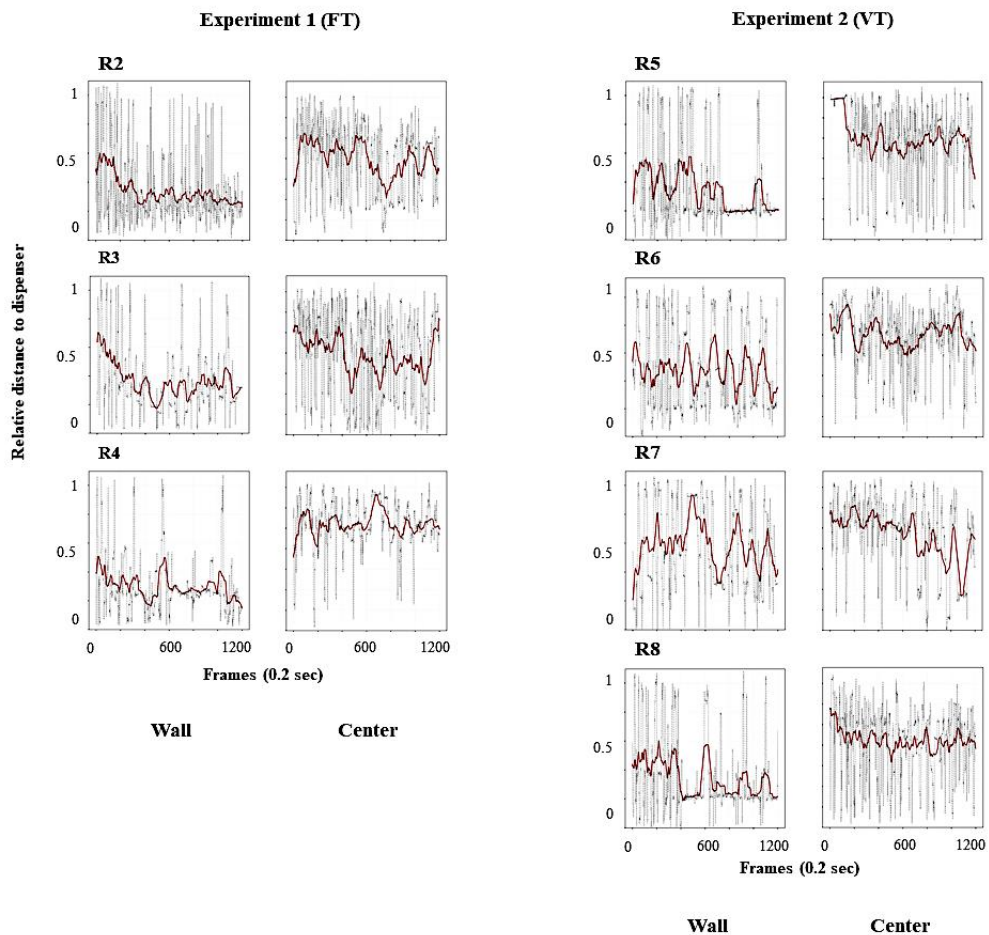
Note. Each panel shows the relative value of the distance (0 = minimum to 1 = maximum) from the rat to the dispenser, every frame or 0.2 s (gray dots) and a moving average of 200 frames (red line) for a complete session. Each row depicts data for one rat, and each column depicts data for sessions 20 (last session of Wall-Condition) and 40 (last sessions of Center-Condition) for Experiment 1 (FT schedule) and Experiment 2 (VT schedule).

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Figure 3 (left side) shows the normalized, moment to moment distance (every 0.2 s) from the location of each rat to the dispenser on the last session for each condition. A value close to 1 means higher distance form the rat to the dispenser and a number close to zero means minimum distance. In Condition I, with the water dispenser located in a wall of the chamber, the distance values remained at low levels, although with some variability along the session. In Condition II, with the water dispenser located at the center of the chamber, the distance values remained at higher levels in comparison to the values obtained when it was located at the wall, with some variability along the session.

Figure 4 (left side) shows accumulated time of stays in each square region from a configuration of 10 x10 defined zones. In Condition I, stays were located almost exclusively close to the wall of the MOFS. In Condition II, stays were distributed among the walls and the center of the chamber.

With the same format of previous figures, the left panel of Figure 5 shows the recurrence plots for subjects under the Fixed Time schedule. Both axes show time against time on a time frame of 0.2 s. These plots depict, with a black mark, the reiteration of the organism in a given location at different times (the intersection X, Y, represents it). If, on the contrary, the rat's location was not reiterated between times (frames), a white mark would be shown. The organism's location is defined as a given position value, for a given frame, within a set of 10 x 10 defined zones, comparing rat's location, frame by frame, throughout the session (for a complete description see León et al. 2020).

There are several aspects to consider in these plots. The densification and alternation of black-white, a checkered pattern, indicates high recurrence; this is periodic returns of the organism to given regions. In checkered patterns, the size of the squares indicates the acceleration of the recurrence patterns. Checkered patterns with relatively bigger squares mean recurrence with lower acceleration, while relatively smaller squares mean recurrence with higher acceleration. Finally, a higher proportion of continuing black means higher stays in a given region, while a higher proportion of white means higher transitions among regions.

Under the wall-dispenser condition, all subjects showed a well-defined checkered-pattern, this is, higher recurrence for the whole session, but with different acceleration (R2 depicts a significantly higher acceleration, this is, well defined checkered pattern with small

Figure 4.

Accumulated time of stays in each of 100 zones of the MOFS for the last session for each rat for Condition I and II for Experiment 1(left panel) and 2 (right panel)
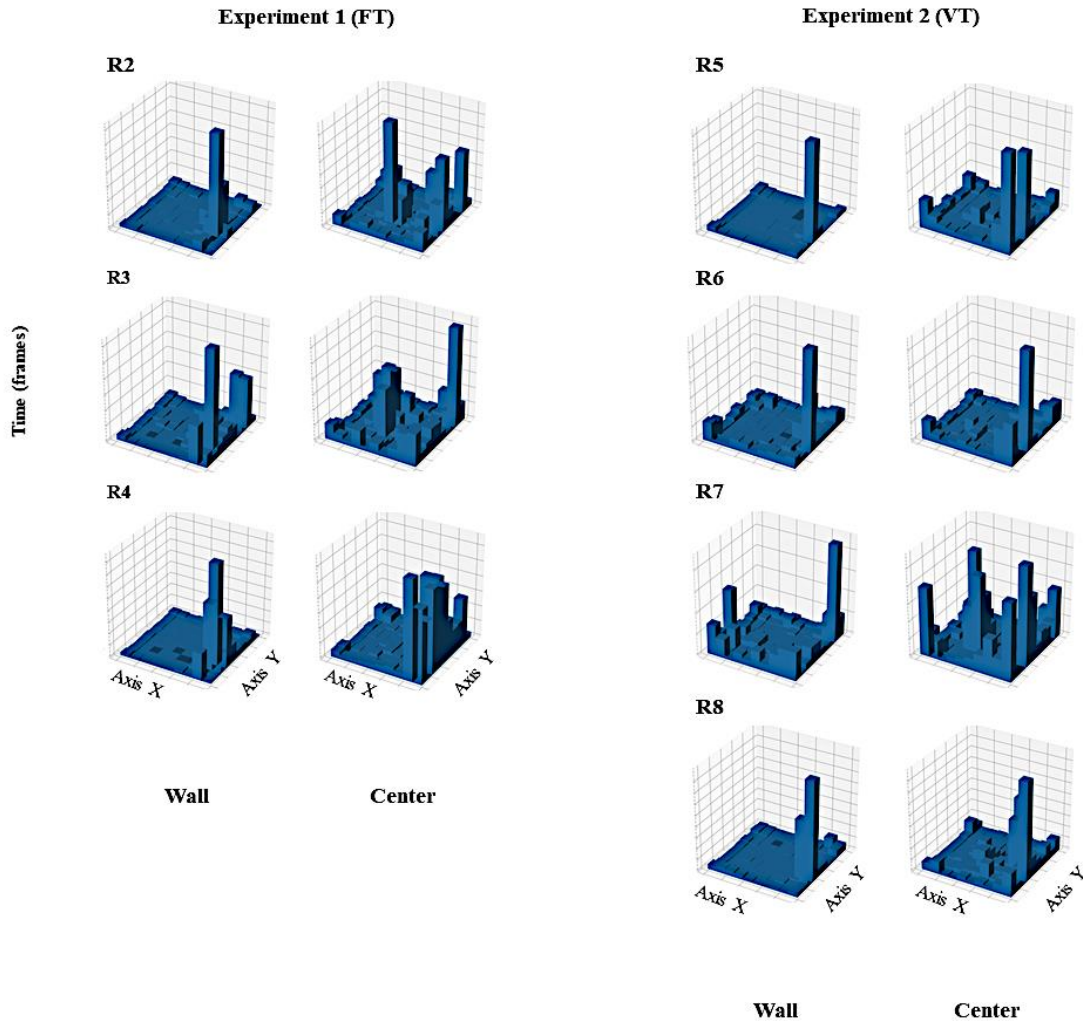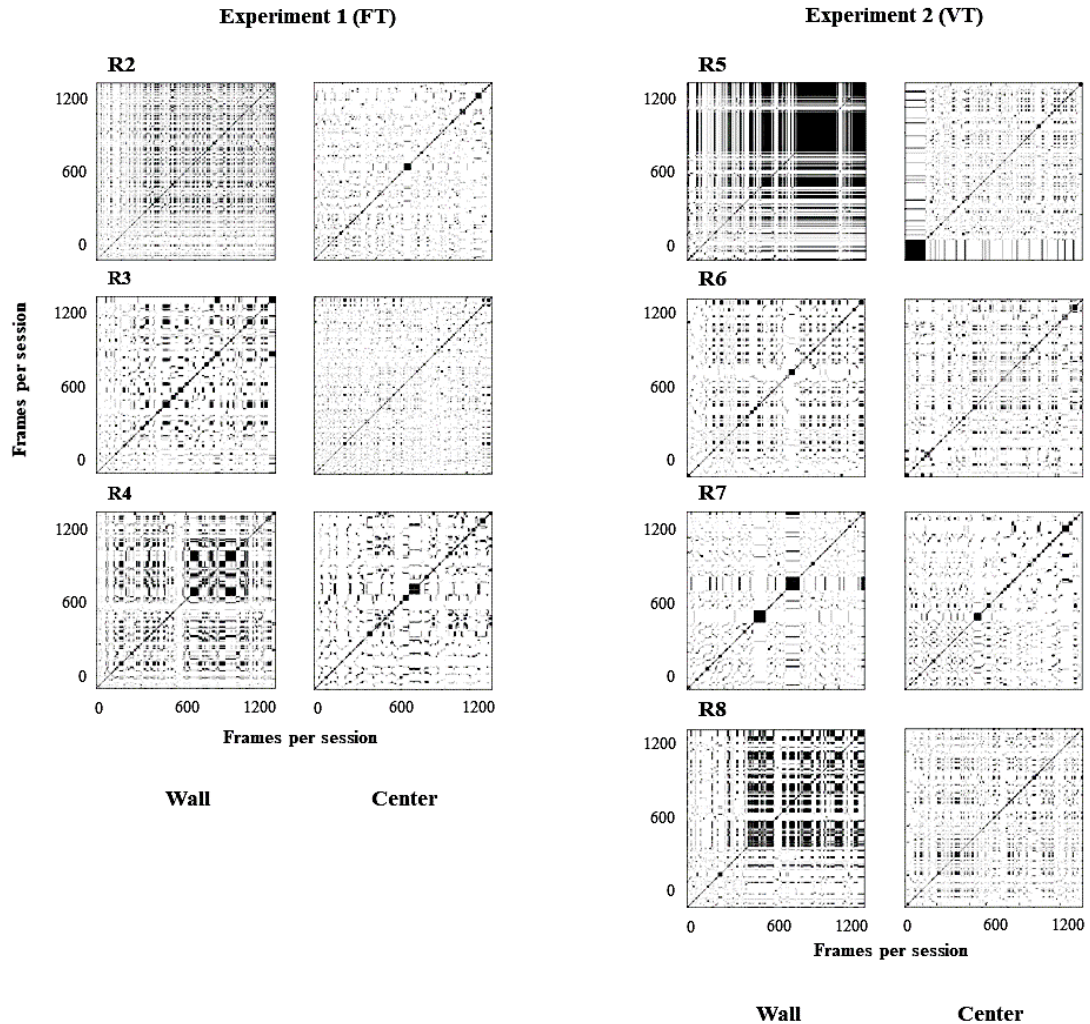

Note. Each panel shows the accumulated time of stays in a square region from a configuration of 10 × 10 zones. Each row depicts data for one rat, and each column depicts data for sessions 20 (last session of Wall-Condition) and 40 (last sessions of Center-Condition) for Experiment 1 (FT schedule) and Experiment 2 (VT schedule).

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Figure 5.

Recurrence plots for the last session for each rat for Condition I and II for Experiment 1(left panel) and 2 (right panel)

Note: Each panel depicts change of regions for each rat in a configuration of 10×10 defined zones every 0.2 s. Each row depicts data for one rat, and each column depicts data for sessions 20 (last session of Wall-Condition) and 40 (last sessions of Center-Condition) for Experiment 1 (FT schedule) and Experiment 2 (VT schedule).

Squares). While, under the center-dispenser condition, checkered patterns were faded and only for brief periods they had a significantly higher acceleration. The predominant quality of recurrence plots, under center-dispenser condition, was the extended white zones which means higher transitions of the organisms among regions with low recurrence and without longer stays (there is not continuing black).

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Figure 6, left panel, shows the entropy of displacement patterns for all rats in the last five sessions in each condition. This measure is a quantitative index of the variation of displacement, high values represents more variation in displacement patterns and low values represents less variation (for the mathematical description of this measure see Appendix C of León et al. 2020 and for its behavioral interpretation see León et al. 2021). We found higher entropy under the center condition ($M = 3.62$, $SD = 0.24$) than for the Wall condition ($M = 3.13$, $SD = 0.66$); $t = 2.77$, N = 30, Effect size dz = 0.92 with 0.98 Power ($\alpha = 0.012$, two tailed). This means that the patterns were more varied with the dispenser at the center.

Figure 6.
Entropy of displacement patterns for all rats in the last five sessions in each condition (wall-center) for Experiment 1
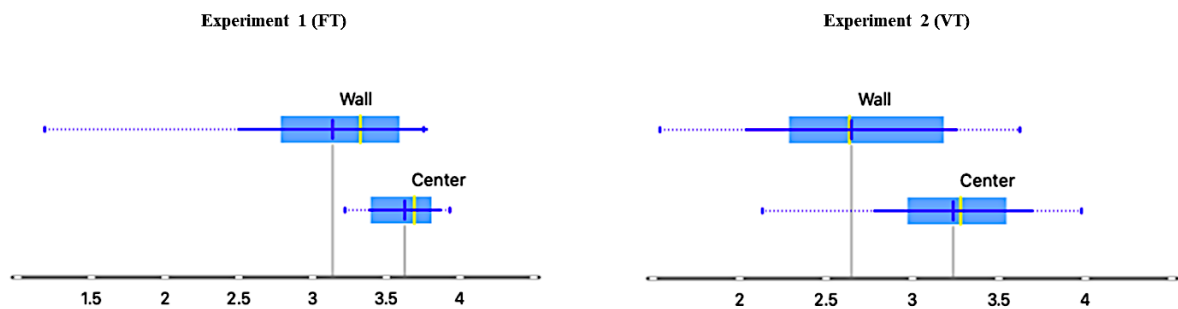
Note: Entropy for all sessions and subjects for Experiment 1 (FT schedule) [t:2.778 (p=0.012, N= 30)], and entropy for all sessions and subjects for Experiment 2 (VT schedule) [t:3.465 (p=0.001, N=40)]. Each box depicts the mean (dark blue vertical line), the median (yellow vertical line), the standard deviation (thin blue line) and the values between the first and the third quartile (blue highlighted area).

Experiment 2

Method

Subjects

Four experimentally-naïve, one male and three female, Wistar rats were used. All rats had the same characteristics of the subjects reported in Experiment 1.

Apparatus

The apparatus was the same as the one described in Experiment 1.

Procedure

The procedure was identical to the one employed in Experiment 1 (see Table 1) with the difference that in Experiment 2 the schedule used was a Variable Time schedule VT 30. The list of values that comprised the VT schedule were 3, 7, 13, 21, 31, 47, 88 s., one value was randomly taken on each occasion from the list without replacement.

Results

Figure 2 (right side) shows rats displacement in the experimental chamber every 0.2 s for a complete session for the VT schedule. The first column depicts routes for the last session of Condition I (wall) and the second column depicts routes for the last session of Condition II (center). The location of the rat in the first 0.2 s of water delivery is highlighted with a black mark. Since the water dispenser remained activated for 3 s, it was still possible that rats contacted the drop of water after the first 0.2s. In Condition I all rats were located close to the dispenser at time of delivery, although there were some crossings between walls for R6 and R7. In that condition, rats location at the time of delivery was close to the dispenser located at the wall. In condition II, for all rats, a back and forth pattern between the walls and center of the chamber was found. The location of the rats at time of delivery was distributed between the wall and the center of the chamber (close to the dispenser).

Figure 3 (right side) shows the normalized, moment to moment distance (every 0.2 s) from the location of each rat to the dispenser on the last session for each condition. moment to moment distance (every 0.2 s) from the location of each rat to the dispenser on the last session for each condition. In Condition I, with the water dispenser located in a wall of the chamber, the distance value remained at low values, although with some variability along the session. In Condition II, with the water dispenser located at the center of the chamber, the distance value remained at higher levels in comparison to the values obtained when it was located at the wall, with some variability along the session.

Figure 4 (right side) shows accumulated time of stays in each square region from a configuration of 10 x10 defined zones. In Condition I, stays were located almost exclusively close to the wall of the MOFS. In Condition II, stays were distributed among the walls and the center of the chamber.

Figure 5 (right side) shows recurrence plots for subjects under Variable Time (Experiment 2). Under the wall-dispenser condition, R5 and R8 showed a heterogeneous session, with an accelerated recurrence-pattern in the beginning of the session, and it transited to a pattern with extended stays (continuing black) from the middle to the end of the session. In contrast, R6 showed a homogeneous session with well-defined recurrence pattern, and R7 depicted horizontal and vertical lines (laminarity), extended transitions among zones (white zones), and few extended stays (black zones). On the other hand, under center-dispenser condition, the change of patterns for R5 and R8 were remarkable; they showed well-defined and accelerated recurrence patterns. R6 showed a very similar checkered-pattern to the one found in the previous condition, but with a smooth acceleration. Finally, R7 showed a pattern with faded laminarity and without extended stays. In brief, in two subjects (R5 & R8), the spatial dynamics increase remarkably in the central-dispenser condition in relation to wall-dispenser condition; and in two subjects, this increase was slight.

Figure 6, right panel, shows the entropy of displacement patterns for all rats in the last five sessions in each condition. In the center condition we found higher entropy ($M = 3.23$, $SD = 0.46$) than for the Wall condition ($M = 2.64$, $SD = 0.62$); $t = 3.46$, N = 40, Effect size dz = 0.82 with 0.95 Power ($\alpha = 0.0015$, two tailed).

Discussion

The purpose of this paper was to conduct a direct replication of León et al. (2020) study, presenting the location of water delivery in a reversed order, this is, first with water located at the wall and then at the center. With this purpose, we compared the spatial behavioral dynamics under two time-based schedules (fixed vs variable time) of water delivery, and two different locations of water delivery (perimetral zone vs. central zone).

Similar to León et al. (2020), we found that, under both schedules (FT and VT) at the moment of water delivery, when the dispenser was at the center zone, the animals were

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usually far and scattered from the dispenser (they were near the perimeter zone), while when the dispenser was in the perimetral zone, the animals were near the dispenser. It was interesting to notice that with the dispenser at the center, back-and-forth patterns associated with the deliveries took place while with the dispenser in the perimeter, patterns of stays took place and distance to the dispenser was significantly reduced. On the other hand, the distribution of time spent in zones throughout the sessions confirmed that, under dispenser at the center condition, two functional segments with associated approach patterns emerged in different physical areas: the zone of water delivery, at the center, and perimeter zone. While, under dispenser at the wall condition, both functional segments, zone water delivery and safety area, converged in the perimetral zone. The routes, distance to the dispenser and time spent in zones, for each experimental condition in both experiments, resulted in well differentiated recurrence patterns and a robust difference in entropy: higher values at the center condition in comparison to the wall condition.

The decrement of spatial dynamics, with the dispenser located in the perimeter, was more salient under the VT schedule than under FT, an expected finding given the literature (Eldridge et al., 1988; Van Hest et al., 1986). Nevertheless under VT with the dispenser at the center, a higher dynamics was observed in all subjects (e.g., back and forth patterns, higher and accelerated recurrence), a finding that could be unexpected if only the stimulus schedule is considered.

The present work provides evidence that the decrement in the dynamic of displacement patterns was due to the experimental arrangement: location of water delivery and schedules of reinforcement, and not to a sequencing effect. As we previously stated, replication is an important process of the scientific practice since it allows to provide robust evidence about the reliability of the effects of our procedures (Baker, 2016). With the results of this study we can confidently state that the decrement in behavioral dynamics observed was a function of the location of water dispenser in combination with the schedule of delivery.

In addition to the replication of previous findings controlling for sequencing effects, the present study replicates and strengthens the following assumptions of Leon et al. (2020a): a) there is a differential ecological segmentation of the experimental arena, regardless of scheduled contingencies; b) the functional relevance of the space on the

dynamics of behavior depends both static (e.g. delimited zones, texture path and dispenser's location) and dynamic (e.g. water delivery and stimuli-schedule) arrangements of the environment; this would be referred to as: functional densification of space ; c) the proposed approach is a comprehensive and allows a broad characterization of the continuum of behavior that is difficult to obtain with approaches based only in discrete responses or other unique measure of the spatial dimension of behavior (Henton & Iversen, 2012); d) the behavior is an integrated functional system comprising an environment-subsystem and an organism-subsystem, in which the ecological relevance of the events and segments of the space are co-determined by the qualities of the organism, defined in a phylogenetic and an ontogenetic way; e) recurrence plots and entropy are useful representations and non-first-order measures in order to characterize, in plausible way, the spatial dynamics of behavior, and its embedded features neglected under standard approaches (León et al., 2021).

Finally, there is pending empirical work for future studies. The current study invites to the parametric study of the effect of the dispenser's location on spatial dynamics of behavior and it's transitions, because in the present work, and in the previous one, only the extreme values of this parameter were explored. Another pending task is the systematic evaluation of the effect of the texturized patch as discriminative segment or signal of water delivery zone.

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