Memoria espacial a largo plazo en humanos entrenados en un laberinto virtual

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Resumen

En este estudio se evaluó la memoria espacial a largo plazo en humanos. Para ello, se empleó un diseño cuasiexperimental en el que se entrenó a tres grupos de estudiantes de pregrado en un laberinto virtual de agua para localizar una plataforma oculta cuya ubicación era señalada por un conjunto de claves. Se realizó un pretest sin plataforma antes del entrenamiento, e inmediatamente después se condujo un postest (Grupo 0 h), así como después de un intervalo de retención de dos días (Grupo 48 h) y siete días (Grupo 168 h). En el pretest no se encontró evidencia de preferencia por alguna zona del laberinto. A lo largo de los ensayos de entrenamiento, el tiempo para encontrar la meta disminuyó sin diferencias entre grupos. Durante el postest, todos los grupos mostraron una preferencia por el cuadrante reforzado, sin embargo, el tiempo de permanencia, la distancia de nado y la precisión de la conducta de búsqueda en dicha zona fue equivalente entre el Grupo 0 h y el Grupo 48 h, aunque mayor a la mostrada por el Grupo 168 h. Estos datos indican cambios ocurridos 48 h después de la adquisición en la memoria espacial a largo plazo en humanos. Se discuten los resultados a partir de procesos generales de memoria y procesos específicos propuestos por teorías particulares de memoria espacial; y al final se abordan las implicaciones clínicas y pertinentes al campo de la psicología comparada.

Palabras clave: Laberinto virtual de agua, retención, olvido espontáneo, memoria espacial.

Long-term spatial memory in humans trained in a virtual maze

Abstract

In this study we evaluated the long-term spatial memory in humans. A quasiexperimental design was used in which three groups of undergraduate students were trained in a virtual water maze to locate a hidden platform whose location was indicated by a set of cues. A pre-test without platform was performed prior to the training, and a post-test was conducted immediately after this (Group 0h), or after a retention interval of two (Group 48h) or seven days (Group 168h). For the pre-test, there was no evidence of preference for any area of the maze. Throughout the training trials, the time to find the goal decreased without differences between groups. During the post-test, all groups showed a preference for the reinforced quadrant, although the spent time, swimming distance, and accuracy of the search behavior in that area was equivalent between Group 0 h and Group 48 h, but higher than that shown by the Group 168 h. These data indicate changes in long-term spatial memory in humans, occurring after an interval of 48 h after its acquisition. The results are discussed on the basis of general memory processes and specific processes proposed by particular spatial memory theories. The clinical and comparative psychology implications are also addressed.

Key words: Virtual water maze, retention, spontaneous forgetting, spatial memory.
Memória espacial de longo prazo em humanos treinados num labirinto virtual

Resumo

Neste estudo, avaliou-se a memória espacial de longo prazo em humanos. Para isso, empregou-se um desenho quase-experimental no qual se treinou três grupos de estudantes de graduação num labirinto virtual de água para localizar uma plataforma oculta cuja posição era sinalizada por um conjunto de chaves. Realizou-se um pré-teste sem plataforma antes do treinamento, e imediatamente depois se conduziu um pós-teste (Grupo 0 h), assim como depois de um intervalo de retenção de dois dias (Grupo 48 h) e de sete dias (Grupo 168 h). No pré-teste, não se encontrou evidência de preferência por alguma área do labirinto. Ao longo dos ensaios de treinamento, o tempo para encontrar a meta diminuiu sem diferenças entre grupos. Durante o pós-teste, todos os grupos mostraram uma preferência pelo quadrante reforçado, contudo o tempo de permanência, a distância de nado e a precisão do comportamento de busca nessa área foi equivalente entre o Grupo 0 h e o Grupo 48 h, embora maior à amostragem pelo Grupo 168 h. Esses dados indicam mudanças ocorridas 48 h depois da aquisição na memória espacial de longo prazo em humanos. Discutem-se os resultados a partir de processos gerais de memória e de processos específicos propostos por teorias particulares de memória espacial; ao final, abordam-se as implicações clínicas e pertinentes ao campo da psicologia comparada.

Palavras-chave: Esquecimento espontâneo, labirinto virtual de água, memória espacial, retenção.

INTRODUCTION

Through spatial memory organisms can acquire, store and retrieve knowledge about the characteristics of the environment and thus draw direct routes between them and the location of useful goals to solve adaptive problems (Postma, Jager, Kessels, Koppeschaar, & van Honk, 2004). As in other types of memory, in the establishment of spatial memory, the processes of acquisition, consolidation, and retrieval of information take place, in which hippocampus (HC) plays a major role (Florian & Roullet, 2004; Goodrich-Hunsaker, Livingstone, Skelton, & Hopkins, 2009; Moser & Moser, 1998). In addition, from the memory systems approach (Squire, 2004), the spatial memory has been considered part of the declarative memory, either as a component of the episodic memory (Morellini, 2013) or as a separate subsystem (Morris, 2013), whose function is to record the spatial context of the events experienced by the organism (Burgess, Maguire, & O’Keeffe, 2002). Since declarative memory is included in long-term memory, this membership is extensible to spatial memory (Demas, Nelson, Krueger, & Yarowsky, 1996; Morris, 2013).

The concept of long-term memory has been defined in various ways (Morris & Mayes, 2004), although a criterion of temporality indicates that the retention of the information contained in this is of at least 24 h (Izquierdo, Medina, Viana, Izquierdo, & Barros, 1999), and may range from weeks to months (Roth, LaDage, & Pravosudov, 2012 and even years (Rutetti, Justel, & Bentosela, 2009). Under these assumptions, the spatial information retained for more than 24 h can be considered long-term spatial memory. Evidence of this type of memory has been obtained with nonhuman animals, such as birds (Mettke-Hofmann & Gwinner, 2003; Roth et al., 2012 Shiflett, Tomaszycyki, Rankin, & DeVogd, 2004; Wilkie & Willson, 1989) and rodents (Carmack, Block, Howell, & Anagnostaras, 2014; Clark, Broadbent, & Squire, 2005; Conejo, Cimadevilla, González-Pardo, Méndez-Couz, & Arias, 2013; Demas et al., 1996; Méndez-Couz, Conejo, González-Pardo, & Arias, 2015) in studies in which, between the training and a test in a spatial task, a retention interval (RI) was interposed whose duration was within the range of days-weeks-months. The data obtained in these studies have shown that birds retain information about the location of a goal for up to six months after its acquisition, without a significant loss of this due to the passage of time (Roth et al., 2011); conversely, rodents gradually lose it with variations according to the task employed, depending on the increase in the duration of the RI (Clark et al., 2005). Notwithstanding the foregoing, in both birds and rodents, the participation of the HC in the establishment of the long-term spatial memory has been confirmed (Clark et al., 2005; Shiflett et al., 2004). On the other hand, although spatial memory has been widely investigated in humans exposed to real and virtual environments (e.g., Chamizo, Aznar-Casanova, & Artigas, 2003; Goodrich-Hunsaker et al., 2009; Hardt, Hupbach, & Nadel, 2009; Jacobs, Laurance, & Thomas, 1997; Schoenfeld, Foreman, & Leplow, 2014; Speiker, Astur, West, Griego, & Rowland, 2012; Ross, Skelton, & Mueller, 2006), studies that address the effect of the passage of time in the retention
of spatial memory, are currently scarce, and the majority of them have been conducted as single case designs that analyze the spatial memory of patients with damage to the medial temporal lobe, including the HC. The overall result of these works has been the absence of long lasting retention of spatial memory acquired in various spatial tasks (Maguire, Nannery, & Spiers, 2006; Rosenbaum, Cassidy, & Herdman, 2015; Rosenbaum, Winocur, Grady, Ziegler, & Moscovitch, 2007; Teng & Squire, 1999; Tramoni et al., 2011). In counterpart, one of these studies reported that healthy population retains the spatial information for several weeks after its acquisition (Tramoni et al., 2011). While research with nonhuman animals and some with humans is consistent with the concept of long-term spatial memory, the diversity of spatial tasks and the use of different RI for the species studied, complicates systematic analysis, which in turn hinders its theoretical development (c.f., Moscovitch, Nadel, Winocur, Gilboa, & Rosenbaum, 2006; Rosenbaum et al., 2007).

The importance of the systematic investigation of the long-term spatial memory is given by its theoretical and applied. In the first case, despite the fact that the Theory of the Cognitive Map (CMT) (O’Keefe & Nadel, 1978; Manns & Eichenbaum, 2009) has been considered one of the most influential approaches in research on spatial memory (Kelly & Gibson, 2007), its approach on the effect of the passage of time in this type of memory is underdeveloped and very little research has been done on it. One of the theses of the CMT is that the passage of time has no effect on spatial memory. Consequently, this theory does not predict differences in spatial memory when evaluated in different RI after acquisition. Although the data obtained with birds are consistent with that prediction, those reported with rodents are not. For humans, current data are insufficient to be able to verify or refute such an approach. The implications in an applied context are linked with the establishment of experimental models for clinical research in memory. There is evidence of a deficit in the episodic memory product of aging, one of its expressions being the difficulty to retrieve information about the context of occurrence of the target event (Chalfonte & Johnson, 1996). Some of that information is provided by spatial memory (Burgess et al., 2002). Thus, validating a methodology for the study of long-term spatial memory in humans, would be useful to describe and compare its characteristics in healthy and clinical populations, as a potential diagnostic tool and even for the evaluation of pharmacological treatments (c.f., Schoenfeld et al. 2014; Spieler et al., 2012).

The water maze (Morris, 1984) is the most widely used spatial task in the research of spatial memory with rodents (D’Hooge & De Deyn, 2001; Vorhees & Williams, 2014), and possibly with humans, using its virtual version (e.g., Jacobs et al., 1997). Additionally, the evidence of neurobiological and behavioral similarities, which is also dependent on HC (Goodrich-Hunsaker et al., 2009; Morris, Garrud, Rawlins, & O’Keefe, 1982; Wooley et al., 2013), in both species, facilitates the comparison of the results obtained in diverse studies. In this task, the subject must swim in a pool to learn the location of a platform hidden below the surface of the water. Later, during a nonplatform test trial, subjects typically show a preference for the area in which it was located. For rodents, usually the training trials are distributed in various daily sessions and the test is done after the final trial or after a 24 h RI (Morris, 1984; Vorhees & Williams, 2014). For humans, training and test trials are usually performed in a single session (e.g., Astur, Ortiz, & Sutherland, 1998; Goodrich-Hunsaker et al., 2009; Hamilton, Driscoll, & Sutherland, 2002; Jacobs et al., 1997; Kolarik et al., 2016; Schoenfeld et al., 2014). The time when the test is performed has theoretical implications given the type of memory evaluated since it has been pointed out that its conduction immediately at the end of training requires the use of short-term spatial memory, while its delayed conduction (≥ 24 h) requires long-term spatial memory (Balda, Efouebe, Lorenzini, & Bucherelli, 2005; Spreng, Rossier, & Schenk, 2002; Vorhees & Williams, 2014).

To verify the contents of both memories, in one study (Balda et al., 2005) rats were trained in daily sessions in the water maze, and in each session they performed a pre and a post-test. In all sessions, the post-test revealed preference for the reinforced quadrant; however, for the pre-test, the same result occurred only in the last sessions. The authors argued for the use of short-term spatial memory during the post-test; while to perform the pre-test a long term spatial memory was required, established only when they advanced in training. These results indicate that performance in an immediate test is not a reliable indicator of long-term spatial memory, at least for rodents. There are currently no human-like studies, which preclude the evaluation of similarities and differences between their short-and long-term spatial memory.

The objective of this study was to describe the effect of the passage of time in spatial memory in humans. Thus, after training in a virtual maze of water (VWM), a group of participants conducted an immediate test trial, while two other groups carried it out in a delayed form (48 and 168 h, respectively). The underlying assumption of this design is that the immediate testing will reveal the contents of the short-term spatial memory, while the delayed tests will reveal the contents of the long-term spatial memory.
Method

Participants

A convenience sampling technique was used to recruit 36 undergraduate students of both sexes and in an age range of 18 to 16 years of age (M = 20.69, SD = 1.99), which were divided into three matched groups for the sex variable (Group 0 h, Group 48 h, and Group 168 h) of 7 women and 5 men each. The participants were naive about the objectives of the experiment and without experience with the task employed or with any similar one. They had a good overall health, normal or corrected vision, and did not consume any drug or substance that would affect their cognitive or motor performance. The performance in the experiment (i.e., learning of the experimental task) did not justify the exclusion of any participant’s data. The collaboration was voluntary and prior to the completion of the study all participants were informed of their rights according to the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). In addition, the execution of the study followed the ethical rules and considerations for research currently in force in Mexico (Sociedad Mexicana de Psicología, 2010).

Apparatus and materials

The experiment was conducted in a room with a chair and a desk on which a 14-inch IBM compatible PC was located, configured with Windows 7, and with a color monitor set at a resolution of 1024 x 768 pixels, on which the experimental task was presented.

Experimental task

The experimental task was designed with the Maze Suite software v. 2.3.0.1 (Ayaz et al., 2011) and consisted of a VWM in which was presented— from a first-person perspective— an octagonal pool filled with clear blue water and surrounded by a gray wall on which several three-dimensional geometric figures (S: Sphere; C: Cube; RP: Rectangular parallelepiped) of different colors (Y: Yellow; B: Blue; G: Green; R: Red) were placed in a distributed manner. The pool was located in a room with walls of different colors and textures, and with a cloudy sky as a ceiling. The room elements and three-dimensional geometric figures were useful cues to locating a hidden platform below the water level, whose size was approximately 3.5% of the total pool area (See Figure 1). Displacement in the VWM was controlled with the direction arrows on the computer keyboard: (↑) Swim forward and (↓) swim back, for the rotation over the axis of the participant was used (←) turn left and (→) turn to the right (Hardt et al., 2009). A turn of 360° took approximately 5 s, while crossing the pool required around 7 s.

Design

It was a quasi-experimental design with several comparison groups and pre-test and post-test measurements. The groups were matched according to the sex variable. After a pre-test, all the groups were exposed to 12 training trials in

Figure 1. Left: Schematic view of the VWM. The inner box indicates the location of the hidden platform (thick line) and the internal sub-quadrant (thin line). The arrows to the center of the walls are the starting points and the letters in the legend together with each open circle point out the three-dimensional geometric figure employed and its color. Right: View of the VWM from the participant's perspective.
the task and subsequently the post-test was carried out. The variable to be manipulated was the RI before the post-test.

Procedure
The experiment was performed individually and comprised five phases: (a) Practice, (b) Pre-training, (c) Pre-test, (d) Training, and (e) Post-test. The Practice phase was useful to familiarize the participants with the displacement in the VWM, and together with the Pre-training phase they allowed to evaluate their motor, motivational and visual ability (c.f., Hamilton, Kodituwakku, Sutherland, & Savage, 2003). In these phases the presentation of the walls of the virtual room, the ceiling, and the keys was omitted. Group 0 h carried out the five phases in a single session of about 15 min, while the group 48 h and the group 168 h carried out the first four phases in a session and the last phase was conducted in a subsequent session. For each phase, at the end of a trial the participants had to press the ENTER key on the computer keyboard to present the next event.

The Practice phase comprised a single trial of 30 s without a platform, the beginning of which went to the center of the VWM, with the north-facing view. Participants were instructed to imagine that they were swimming in a pool and were then asked to move freely in it. Subsequently, a Pre-training phase was carried out with two trials, which was useful to teach participants to locate a visible platform in the VWM. They were asked to move in the environment until they contacted a visible platform on the water level and located in a pool area. Each trial of this phase began at the center of the northeast quadrant of the VWM, and a visible platform was presented on the water level, located in front of the central part of the north wall (first trial) or the south wall (second trial). One trial ended when the participant made contact with the platform and at the center of the monitor appeared a legend that said "Here is the platform" (positive feedback). After this phase was concluded, participants were told that in subsequent trials the platform would be hidden below the surface of the water level and that, in order to avoid drowning, they had to find it before a time limit had elapsed. It was also mentioned that various elements of the environment could help them determine the position of the platform. After clarifying the pertinent doubts the researcher abandoned the experimental environment and the participant was able to begin with the pre-test. This phase included a 60 s trial without platform. The participant started the trial in front of the south wall of the pool and, due to the absence of the platform, at the end of the test, a message was sent to the center of the monitor that read "You died" (negative feedback). Immediately after the Training phase began, which included 12 trials with a maximum duration of 60 s, with the hidden platform present, located in the northwest quadrant. Each trial began at a different cardinal point, programmed semi-randomly in order not to present the same in a consecutive way, and with the view of the participant oriented towards the wall of the VWM. At this phase, if the participant located the platform, positive feedback was shown; in case of exhausting the duration of the trial without finding it, the negative feedback was shown. Once the relevant feedback was given, the following trial or phase was scheduled according to the case. Finally a post-test was conducted, with the same characteristics as the pre-test. Group 0 h carried out the post-test immediately after the Training phase; in contrast, the participants of Group 48 h and Group 168 h were quoted by the investigator to complete the experiment two and seven days later, respectively. For these last groups, during the final session the participants were seated in front of the computer whose monitor showed the legend "please locate the hidden platform as you did before." The post-test started after pressing the ENTER key on the computer keyboard. For all groups, after this phase, the experiment concluded.

Behavioral indicators
The following behavioral indicators were recorded:
1. Escape latency: Time elapsed from the start of the trial to locate the hidden platform.
2. Time spent in reinforced quadrant: Time elapsed in the quadrant of the VWM in which the hidden platform was located. Although this is the most commonly analyzed measure in studies using the water maze, its validity has been questioned (e.g., Hardt et al., 2009), so it has been suggested to use areas of ad hoc analysis, generally smaller, to reveal in more detail the quality of the spatial memory (i.e., subcuadrantes; c.f., Blokland, Geraerts, & Been, 2004).
3. Internal sub-quadrant: An area peripheral to the platform location, which accounted for 37% of the total area of the reinforced quadrant and excluded a band adjacent to the perimeter of the quadrant (see Figure 1).
4. Precision Index (PI): The proportion of the time remaining in the internal sub-quadrant relative to the time elapsed in the reinforced quadrant. It is obtained by dividing the time of permanence in the sub-quadrant between the permanence in the reinforced quadrant. The result has a range of 0 to 1, and indicates the area where the platform was preferably sought: with PI > .5 greater search in the internal sub-quadrant (greater precision), with PI < .5 greater search in the peripheral zone of the reinforced quadrant (less precision), with PI = .5 the search was indistinct between these two areas. Its use caters to the need to evaluate the
precision in the search behavior in spatial tasks rather than the permanence in specific zones (c.f., Kolarik et al., 2016).

5. Swimming distance in the VWM and in the reinforced quadrant: Length in virtual units (vu) of the route traveled when searching for the hidden platform.

Similar to other studies (e.g., Astur, Purton, Zaniewski, Cimadevilla, & Markus, 2016; Kolarik et al., 2016), these variables were considered as an indicator of the motivational level of the participants during the test. The indicator 1 was used in the training phase, whereas indicators 2, 2 and 4 were used in both tests, and 5 was only used in the post-test.

Data analysis

Data analysis was conducted with the SPSS v. 20. Initially the normality of the data was assessed using the Shapiro-Wilk test, and subsequent analyzes included t tests of a tail for a group, and analysis of variance (ANOVA), with the post-hoc Tukey's Honest Significant Difference test. These two tests are robust tests against the normality assumption, in such a way that they guarantee the certainty of their results even with deviations from the assumption of normality, as was the case for some of the variables analyzed in this study, although caution should be taken with the inferences made. In addition, both tests are considered a better option for data analysis, even with groups of n < 5, versus nonparametric tests (Khan & Rayner, 2003; de Winter, 2013). The d of Cohen and the partial eta-squared were calculated as effect-size indicators, considering a small, medium and large effect a d ≥ .20, .50, and .80, and a ηp² ≥ .01, .06, .14, respectively (Aron & Aron, 2001). For all tests, a result was considered significant at a value of p ≤ .05.

RESULTS

This section presents the results on the normality of the data and the initial equivalence of the groups during the practice phase and the pre-training phase. Subsequently, the results obtained during the training are presented, and finally the obtained ones are compared during the pre-test and the post-test.

Normality of the data and initial equivalence of the groups.

The Shapiro-Wilk test showed evidence of the normality of the data (p > .05) in all groups for the variables permanence in reinforced quadrant, swim distance in the VWM and in the reinforced quadrant, and PI. For permanence in the internal sub-quadrant, this distribution was shown in Group 48 h in the pre-test, and in groups 0 h and 168 h in the post-test. For the training, a greater number of tests per group did not meet the requirement of normality (p < .05). Despite the foregoing, the parametric analysis described above was undertaken, given the advantages of this one on nonparametric analysis, even under the reported conditions. In addition, a preliminary analysis showed no difference between groups in the swimming distance in the VWM during the practice and pre-training phase, or in the latency to reach the visible platform, when the trials of this last phase were collapsed in a single block (p > .05).

Performance during training.

Figure 2 shows a systematic and undifferentiated decrease between groups for escape latency, which was confirmed by conducting an ANOVA 3 x (12) with the groups (0 h vs. 48 h vs. 168 h) and trials (1-12) factors. This analysis revealed a major effect for the trials factor, F(11, 363) = 26.13, p < .01, ηp² = .44, with the performance in Trial 1 other than that of Trial 8 (p < .01).

Comparison of the performance of the groups during the pre-test and the post-test.

For all groups, the permanence in the reinforced quadrant was lower during the pre-test as compared to the post-test (See Figure 3). An ANOVA 3 groups (0 h vs. 48 h vs. 168 h) x 2 tests (pre-test vs. post-test) revealed a major effect for each factor and its interaction, groups: F(2, 66) = 3.74, p < .01, ηp² = .10; tests: F(1, 66) = 53.35, p < .01, ηp² = .44; Groups x Tests: F(2, 66) = 3.29, p < .05, ηp² = .09. This interaction was analyzed in two ways, each using one-way ANOVA. In the first analysis, the performance was compared between groups during each test. No differences were detected for the pre-test (p > .05), although there were for the post-test, F(2, 33) = 5.07, p = .05, ηp² = .23.
Group 0 h different from Group 168 h ($p < .01$). No other comparison was significant ($p > .05$). In the second analysis the performance between tests was compared by group, which was significantly different ($p < .01$) in all the cases, Group 0 h: $F(1, 22) = 29.79, \eta_p^2 = .57$; Group 48 h: $F(1, 22) = 16.73, \eta_p^2 = .43$; Group 168 h: $F(1, 22) = 8.08, \eta_p^2 = .26$.

Additionally, $t$-tests showed during the post-test a spent time above the randomness level (15 s) in the reinforced quadrant for all groups (See Table 1).

![Figure 3](image)

**Figure 3.** Time spent in reinforced quadrant. Error bar indicates the standard error of the mean. ** = $p < .01$.

The ANOVA of 3 groups x 2 tests described above was also used to analyze the permanence in the internal sub-quadrant, in which the permanence was also lower during the pre-test compared to the post-test for all the groups (See Figure 4). This analysis revealed a main effect ($p < .01$) for each factor, groups: $F(2, 66) = 6.23, \eta_p^2 = .15$; tests: $F(1, 66) = 77.39, \eta_p^2 = .54$, being the permanence of the group 168 h in this area different from the Group 0 h ($p < .01$) and the Group 48 h ($p = .05$). No other comparison proved significant ($p > .05$). Except for Group 168 h, the permanence during the post-test in this same area exceeded the chance level, which was 8.97 s (See Table 2).

![Figure 4](image)

**Figure 4.** Time spent in internal subquadrant. Error bar indicates the standard error of the mean. ** = $p < .01$.

The PI is shown in Figure 5. A one-way ANOVA showed differences between groups, $F(2, 33) = 3.64, p < .05, \eta_p^2 = .18$, being Group 48 h other than group 168 h ($p < .05$). No other comparison proved significant ($p > .05$). $t$-tests showed that the point of indifference for PI was only exceeded by Group 0 h, $t(11) = 2.92$ ($d = 1.76$), and Group 48 h, $t(11) = 4.41$ ($d = 2.65$), $p < .01$.

Finally, the swimming distance traveled in the VWM and in the reinforced quadrant was higher during the post-test compared to the registered for the pretest in the same areas. This is shown in Figure 6 and Figure 7, respectively. For the first case, this was confirmed by an ANOVA 3 groups x 2 tests which revealed a main effect for the last factor, $F(1, 66) = 11.70, p < .01, \eta_p^2 = .15$. For the second case, the analysis described above showed a main effect for each factor and its interaction, groups: $F(2, 66) = 12.65,

### Table 1

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<td>25.37 (10.37)</td>
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<td>21.01 (7.54)</td>
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Note: ** $p < .01$, one tail

### Table 2

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<td>48 h</td>
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<td>1.82</td>
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<tr>
<td>168 h</td>
<td>11.00 (5.76)</td>
<td>1.22 n. s.</td>
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Note: ** $p < .01$, one tail; n. s. = no significant
p < .01, $\eta_p^2 = .27$; tests: $F(1, 66) = 59.55$, $p < .01$, $\eta_p^2 = .47$; Groups x Tests: $F(2, 66) = 9.15$, $p < .01$, $\eta_p^2 = .21$. For the analysis of the interaction, initially two one-way ANOVA were conducted, in order to compare between groups the performance during each test. Although no differences were detected for the pre-test ($p > .05$), these were found for the post-test, $F(2, 33) = 15.48$, $p = .01$, $\eta_p^2 = .48$, being Group 0 h different from Group 48 h ($p < .05$) and Group 168 h ($p < .01$), and Group 48 h different from Group 168 h ($p = .01$). Subsequently, three one-way ANOVA were conducted to compare the performance between tests by group. Significant differences were reported in all cases, $F(1, 22) = 55.32$ ($\eta_p^2 = .48$), $13.56$ ($\eta_p^2 = .38$), $p < .01$, for Group 0 h and Group 48 h respectively; Group 168 h: $F(1, 22) = 4.59$, $p < .05$, $\eta_p^2 = .17$.

**DISCUSSION**

The objective of this research was to describe the effect of the passage of time on spatial memory in humans. The data obtained show that after its acquisition, the spatial memory was retained without significant changes for a period of up to 48 h. When the time frame was 168 h, it was modified in its content which was reflected in the less persistent and precise search of the goal at the site where it had been previously found.

The performance of the participants during the first phases of the study (i.e., practice and pre-training) demonstrated the relevant motor and visual ability to navigate properly in the VWM. Initial lack of knowledge about the location of the platform was evident due to the lack of preference for an environment quadrant, recorded during the pre-test, as well as the prolonged escape latency in the first trial of the training phase. However, the reduction in this variable over the course of the remaining training trials showed the establishment of spatial memory. This was confirmed by the performance of Group 0 h during the post-test, in which the preference for the reinforced quadrant was detected. These results are consistent with those obtained in other studies that used the traditional water maze, with rodents (e.g., Baldi et al., 2005; Blokland et al., 2004; Clark et al., 2005; Morris, 1984) and the virtual one with humans (e.g., Astur et al., 1998; Goodrich-Hunsaker et al., 2009; Hamilton et al., 2002; Jacobs et al., 1997; Kolarik et al., 2016; Schoenfeld et al., 2014).

The preference for the reinforced quadrant during the post-test, recorded in all the groups, indicates that the
spatial memory in human is retained and it is possible to be recovered until seven days after its acquisition. These data are consistent with the concept of long-term memory (Izquierdo et al., 1999; Roth et al., 2011), and specifically with the long-term spatial memory (Demas et al., 1996; Morris, 2013). A similar effect had been previously demonstrated in rodents (Méndez-Couz et al., 2015) although opposite data were found in another study (Carmack et al., 2014).

The detailed analysis of the performance during the post-test showed the absence of differences in the permanence in the reinforced quadrant between the Group 0 and the Group 48 h. This suggests that the content of short-term and long-term spatial memory is analogous, if not equivalent, at least for a period of up to 48 h. However, the permanence in the reinforced quadrant was higher in Group 0 h compared to that registered for Group 168 h. This inverse relationship between permanence in the reinforced quadrant and the prolonged duration of the RI is similar to that obtained in other works with rodents (e.g., Clark et al., 2005) and implies that, through a lapse of RI, the information contained in the short-term and long-term spatial memory is not equivalent. The contrasting data of Group 48 h and Group 168 h, with respect to Group 0 h, indicate that the moment in which the change occurs is after 48 h. Future studies must confirm that statement.

The analysis of the permanence in the internal sub-quadrant is useful to clarify the way in which the long-term spatial memory could be modified, according to the passage of time. The absence of differences in this variable between Group 0 h and Group 48 h reinforces the aforementioned idea that the content of the short and long temporal spatial memory is similar, at least in the first few hours. However, both groups were different in the mentioned variable, with respect to Group 168 h. Since the permanence in the internal sub-quadrant is an indicator of the accuracy of spatial memory, and the associated search behavior, it is possible to assume that one of the adverse effects of the passage of time is the loss of accuracy in that memory. This is a result commonly reported in memory studies (e.g., Odinot & Wolters, 2006). In favor of this interpretation is the fact that the PI of the Group 168 h reached the point of indifference, which indicates that its search for the platform was performed equally in the internal sub-quadrant as in the peripheral area to this, although still in the reinforced quadrant. The above did not occur with the other groups, which carried out a more precise search of the goal, mostly in the area of the internal sub-quadrant.

The loss of accuracy in long-term spatial memory could be due to general memory processes, specifically to retention and recovery, or to particular processes characteristic of spatial memory. For the first case, it is feasible to propose that the Group 0 h and the Group 48 h acquired (training phase), retained (RI) and recovered (post-test) spatial memory without problems. On the other hand, it was possible that the Group 168 h presented a problem in one of the last two processes, or even in both. From this assumption two explanations are derived. The first one is based on an effect of spontaneous forgetting (Sara, 2000), caused by the prolonged duration of the RI employed in the Group 168 h. According to this explanation, the participants of that group would have been unable to retain the spatial information needed to direct their conduct search to the site where the hidden platform was previously located. The second explanation proposes the occurrence of a problem in the retrieval of information. This implies that information about the location of the platform was maintained during the post-test but the participants were unable to retrieve it. It is even feasible to suppose a mixture of both explanations. A third explanation implies improvement in the memory of the location of the goal as a function of the passage of time, possibly due to a spatial memory consolidation effect (c.f., Ferrara et al., 2006). If this happened, the participants would have been directed to the exact site in which the goal was located and by not finding it there, tended to move to other areas of the VWM in their search (c.f., Hardt et al., 2009).

Although these three explanations are potentially plausible, the current data do not allow any of them to be verified or refuted, so future studies should be expressly designed for that purpose. For the second case, the one referred to particular processes of the spatial memory, the data of the Group 0 h and of the Group 48 h can be explained from the CMT (O’Keefe & Nadel, 1978; for application in humans see Hardt et al., 2009) by supposing that during the training phase the participants formed a mental representation or cognitive map of the VWM.

This representation would have been useful to move directly from the starting point to the goal, and thus, look for it in the reinforced quadrant when it was omitted during the post-test. However, the performance reported by the Group 168 h cannot be explained by the same theory. According to CMT, the passage of time has no effect on the cognitive map. In this way, the performance of that group should have been equivalent to that of the remaining groups. An alternative explanation for this last result would be from the assumptions of the Multiple trace theory (MTT; Moscovitch et al., 2006) and the Hypothesis of memory transformation (HMT; Winocur, Moscovitch, & Sekeres, 2007). In general terms, these two approaches propose that
a memory originally dependent on the HC (as is the spatial memory) can, over time, become an independent memory of that structure. With this transformation, memory loses specific information (e.g., context or other vivid details) of the target event, preserving only its general aspects. Then, one possibility is that the spatial memory of the participants of the Group 168 h, initially rich in details, would have been modified, thus losing specific information about the location of the platform, although preserving the general remembrance of its location in the VWM. This could explain the preference for the reinforced quadrant and at the same time a PI equivalent to the point of indifference.

The set of results obtained has different implications. At the theoretical level and as previously discussed, they reveal the inability to be explained by one of the most relevant theories in the area: the CMT (O’Keefe & Nadel, 1978). This suggests the need to integrate into it, and in related models (e.g., Burgess, Jackson, Hartley, & O’Keefe, 2000), retention and recovery mechanisms that describe the effects of the passage of time on spatial memory. On the other hand, while the MTT (Moscovitch et al., 2006) and HMT (Winocur et al., 2007) approaches are able to offer a plausible explanation, the empirical verification of this is required. For comparative psychology issues, these results reveal similarities with those obtained with rodents when using RI similar. This demonstrates the participation of spatial memory retention mechanisms shared between species. Perhaps the most relevant involvement of our study is in an applied context. Data show the relevance of the environment, design, and behavioral indicators recorded to study the changes that occur in spatial memory depending on the passage of time. This makes our VWM and procedure a potentially useful methodology for its application in healthy and clinical populations, which could provide new knowledge about the typical and pathological function of episodic memory of humans in previously unexplored aspects (e.g., precision) (Kolarik et al., 2016).

Finally, it should be pointed out that there are some aspects that future studies should address in order to further investigate long-term spatial memory in humans. These include gender-related differences in spatial memory (Jones & Healy, 2006) and the differential effects of the use of massive training against dosing training in the water maze (Commins, Cunningham, Harvey, & Walsh, 2003).

In conclusion, data of this study demonstrate the presence in humans of a long-term spatial memory, which is similar to short-term spatial memory, at least up to 48 h after its acquisition, and then modified and causing a change in the associated search behavior.

REFERENCES


