Spatial Memory of Food Location and the Expression of Synapsin I in the Hippocampus of Pigeons (C. Livia)

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Summary

Experience with environmental space triggers behavioral, cellular and molecular alterations in the hippocampus, which includes induction of transcriptional mechanisms that underlies the formation and consolidation of spatial memory. This study investigated acquisition and consolidation of spatial memory in a food choice situation and patterns of expression of the Synapsin I protein in the hippocampus of pigeons after short- and long-duration training. Male adult pigeons received two (short duration) or 7 sessions (long duration) of training in a food choice situation. Each session had 6 trials in a circular arena with 4 feeders, only one containing food. The choice response was defined as orientation, approach and peck at one feeder. Spatial strategy was tested in one session with feeders removed from the arena. Immunohistochemical analysis of Synapsin I protein was conducted in the hippocampus tissue of pigeons in each group. The latency of the choice response of pigeons trained during 7 days decreased across the training sessions. The number of correct choices increased as function of training, varying between 50% and 88%, in the first and seventh session, respectively. The ANOVA for repeated measures indicated significant differences between groups ($p<0.001$) and sessions ($p<0.001$). The cell counts for Synapsin I-positive cells were higher in the hippocampus of E7 pigeons than in the hippocampus of E2 and Control groups ($p<0.05$). The increases in Synapsin-I expression in response to training can be thought as evidence of playing in long-term excitability within synaptic circuits in the hippocampus.

Key words: Spatial memory, hippocampus, Synapsin I protein, neuroplasticity, pigeons.

Memoria espacial de la localización alimentar y la expresión de Synapsin I en el hipocampo de Palomas (*C. livia*)

Resumen

La experiencia con el ambiente provoca alteraciones en el hipocampo, que incluye la inducción de mecanismos de transcripción que subyace a la formación y consolidación de la memoria espacial. Este estudio investigó la adquisición y consolidación de la memoria espacial en situación de elección de alimentos y los patrones de expresión de la proteína Synapsin I en el hipocampo. Palomas adultas machos recibieron 2 o 7 sesiones de una situación de elección de alimentos. Cada sesión tenía 6 ensayos en una pista circular con 4 alimentadores, sólo uno tenía alimento. La respuesta de elección se define como la orientación, el enfoque y picotear en un alimentador. Estrategia espacial se puso a prueba en una sesión con alimentadores retirados de la arena. El análisis de la proteína Synapsin I se llevó a cabo en el tejido del hipocampo de palomas en cada grupo. La latencia de la respuesta de elección de palomas entrenadas durante 7 días disminuyó a través de las sesiones de entrenamiento. El número de elecciones correctas aumentó en función de la formación, que varía entre 50% y 88%, en la primera y séptima sesión, respectivamente. ANOVA para medidas repetidas indicó diferencias significativas entre los grupos ($p<0,001$) y sesiones ($p<0,001$). Los recuentos de células para las células positivas Sinapsina I fueron más altos en el hipocampo de E7 que en el hipocampo de los otros grupos ($p<0,05$). Los aumentos en la expresión de Synapsin-I en respuesta al entrenamiento pueden ser considerados la prueba de la participación en excitabilidad sináptica a largo plazo dentro de los circuitos de hipocampo.

Palabras clave: memoria espacial, hipocampo, proteína Synapsin I; neuroplasticidad, palomas.

Introduction

Many studies provide evidence supporting the assumption that the hippocampus plays an essential role in learning and memory by primates (Eichenbaum, Otto, & Cohen, 1992; Shiflett, Smulders, Benedict, & DeVoogd, 2003) and rodents (Guzowski, Setlow, Wagner, & Mcgaugh, 2001; Kim & Fanselow, 1992; Rolls, 2013; Sprick, 1995; Tischmeyer & Grimm, 1999), as well as in birds (do Amaral-Toma & Ferrari, 2004; Brito, Brito, & Ferrari, 2006; Colombo & Broadbent, 2000; Faria, Sartori, Canova, & Ferrari, 2013; Fremouw, Jackson-Smith, & Kesner, 1997; Mayer, Watanabe, & Bichof, 2010; Reis, Schenka, Melo, & Ferrari, 1999; Watanabe & Bischof, 2012). These studies emphasize that the hippocampus have a critical role in behavioral regulation and cognition, especially in relational types of learning (Eichenbaum et al.) and the spatial and contextual memory requiring map solution (Abel & Lattal, 2001; do Amaral-Toma & Ferrari, 2004; Antoniadis & McDonald, 2000; Fremouw, Jackson-Smith, & Kesner, 1997; Watanabe, Kudoh, Ohnishi, & Shibuki, 2001; Zhou, Xiong, Zhang, & Ge, 2013).

Although the preponderance of studies on spatial memory has been conducted with rodents and primates it was shown that birds are also able to solve spatial memory tasks (do Amaral-Toma & Ferrari, 2004; Fremow et al., 1997; Mayer et al., 2010). Accordingly, pigeons are able to find the position of food in a dry version of Morris

water maze and both the acquisition and retention of the location of hidden food in the maze are impaired by hippocampal lesions (do Amaral-Toma & Ferrari; Fremow et al.). Studies conducted with Zebra finches showed that these birds use a combination of both extra-cues and cage cues to locate food and, thus, provide evidence of the use of spatial maps (Watanabe, Kudoh, Ohnishi, & Shibuki, 2001). Besides, zebra finches that have learned the spatial task showed higher activation of immediate early genes (IEGs) during spatial learning than those that did not rely on spatial cues to find food (Bischof, Lieshoff, & Watanabe, 2006).

The experience-dependent activation of different molecular events and signaling cascades in the hippocampus has been shown in numerous studies have shown that experiences. The activation of these cascades in the hippocampus is essentially initiated by glutamatergic receptors particularly NMDA receptors, followed by the recruitment of second messenger systems, which ultimately leads to the activation of transcription and translation of proteins required for functional and structural changes in the neuron (Kandel, 2001). IEGs are rapidly activated by neuronal activity in different brain areas, particularly in the hippocampus and are supposed to play a crucial role in the transformation of short term enhancement of synaptic efficiency into long-term efficiency changes, which underlies the formation of short-term and long-term memory (Guzowski et al., 2001; Jones et al., 2001; Kubik, Miyashita, & Guzowski, 2007; Lanahan & Worley, 1998; Mayer et al., 2010). Synapsin I is one phosphoprotein implicated in neurotransmitter release, axonal elongation, and formation and maintenance of synaptic contacts (Brock & O'Callaghan, 1987; Gómez-Pinilla, So, & Kesslak, 2001; Jovanovic, Czernik, Fienberg, Greengard, & Sihra, 2000), and can also be considered as a marker to evaluate synaptic function associated with learning and neuroplasticity.

In the last two decades we have seen a growing interest toward a better comprehension of the role of avian hippocampus in cognition and learning, including in spatial tasks (Mayer et al., 2010; Watanabe et al., 2001). The use of pigeons in studies concerned with these issues has been proved to be very appropriate. There has been settled that the mammalian hippocampus has a feed-forward tri-synaptic circuit responsible for memory consolidation (Amaral & Witter, 1989) and a relative consensus can be found in avian species as well (Atoji & Wild, 2006; Kahn, Hough II, Eyck, & Bingman, 2003). The functional, morphological, and anatomical similarities between avian and mammal hippocampus have been repeatedly emphasized. The hippocampus (Hp) of pigeons is a medially situated structure, which extends at the dorsal rim of the lateral ventricles and around the posterior poles of the hemispheres (Karten & Hodos, 1967). Its homology with the mammalian hippocampus complex has been recognized based on criteria of hodology (Kitt, Lee, Fink, & Calvanico, 1986; Krayniak & Siegel, 1978) embryologic development (Puelles & Rubenstein, 1993; Rubenstein & Puelles, 1994), neurochemical organization (Erichsen, Bingman, & Krebs, 1991; Krebs, Erichsen, & Bingman, 1991), neural connections and neurotransmitters (Casini, Bingman, & Bagnoli, 1986; Rosinha, Ferrari, & Toledo, 2009). Besides, studies in our laboratory have demonstrated the involvement of the pigeon hippocampus in fear conditioning

(Reis et al., 1999; Brito et al., 2006, Brito, Britto, & Ferrari, 2011) and in spatial location of food task (do Amaral-Toma & Ferrari, 2004).

The objective of the present study was to determine whether short- and long-duration training in a task of food location induces different patterns of expression of Synapsin I protein in the hippocampus of pigeons.

Methods

Animals

Adult male pigeons (*Columba livia,* age around 18-24 months, weighing on average 450 g, were used in the present study. The pigeons were attributed to seven groups: Experimental 2 (E2; n = 12) birds with short duration training of food location in two 2 sessions; Experimental (E7; n = 14) birds with long duration training of food location in 7 sessions; Control 2 (C2; N = 7) birds exposed to the arena with empty feeders (2 sessions); Control 7 (C7; N = 7) birds exposed to the arena with empty feeders (7 sessions); Näive group (Naïve: N=7), birds that were handled, transported to the laboratory, weighed and carried back to their home cage. The experimental protocol was approved by the Ethics Committee for Animal Experimentation of the Institute of Biology, UNICAMP, Brazil (Protocol #2096-1).

Procedures

› Adaptation to the vivarium conditions

For at least fifteen days before the experiment, the pigeons remained in the vivarium under light-dark cycle of 12:12 h (lights on at 6:00 pm), to adapt to those conditions. In the four days that preceded the beginning of the experimental sessions the animals of all groups were handled at time scheduled for experiment, between 8:00 and 9:00 a.m.

› Weight control and food deprivation

At the end of the adaptation period, the pigeons were weighed daily during 7 days in order to calculate the average *ad libitum* weight (average 450g to 550g) and the experimental weight (80% of the *ad libitum* weight) the birds underwent food deprivation, with *ad libitum* water, consisting of a gradual reduction of the daily food supply, until achieve experimental weight. This was accomplished between 7 - 10 days after initiation of food deprivation.

Apparatus

All tests were conducted in a wood circular arena with 1.5 m diameter and 50 cm high, with the floor and the wall painted white. The floor was covered with a rough brown paper that was changed in each session. It was positioned in the center of a room (2.11 m wide x 3.10 m length x 2.77 m height) that had one entrance door, visual stimuli like sockets, light switches, and in each wall there was a picture (30 cm x 40 cm) -a red circle, a blue square, a green triangle or a yellow star– which provided distal landmarks.

Four points equally spaced along the circumference of the arena were arbitrarily defined as: N (north), E (east), S (south) and W (west). They served as orientation points every time the pigeon was placed inside the chamber. The area of the arena was also virtually divided into four equal sized quadrants (NE, SE, SW and NW). In each quadrant there was one identical ceramic cup. Located at the SW quadrant there was a cup filled with food was covered with sand whereas in the other three quadrants the cups had only plain sand. All the sessions were recorded with a digital camera (Seykon, Mod. SK 1710-c) located

2.60 m above the center of the experimental arena and connected to a monitor and computer system located in an adjacent room.

› Habituation to the experimental chamber

Pigeons had 20 min sessions of habituation to the experimental arena in two days prior to the first training session. The habituation session consisted in two 10 min exposures to the arena that were separated by a 5 min interval. This interval was spent in a cage located at an adjacent room. During those two sessions the arena was without the food cups.

› Training in spatial food location

The spatial training was conducted during two or seven sessions, with six trials in each session. The location of the correct food cup remained fixed during all the six trials - in the center of N quadrant, 18 cm from the arena wall, but in each trial the bird was liberated at a different starting point (N, E, S, or W). Before the beginning of each trial the light in the room was turned off and the pigeons were gently placed in the arena with its head facing the wall of the arena. The lights and a digital chronometer were simultaneously turned on and the latency of the choice response and the accuracy of the choice (correct response, error or omission) were recorded by the one experimenter. The latency was recorded when the pigeon first exhibited a first pecking response in one of the food cups, which was defined as the choice response. The choice was rated as correct if the pigeon pecked the cup containing food in the SW quadrant, as error if the pigeon first pecked a cup containing only sand in any of the other quadrants and as omission if no choice response was emitted in the 5 min trial. After a correct choice the pigeons were allowed access to the food during 5 s. The end of the trial was signaled by lights off that occurred after this feeding period or that followed one wrong choice or one omission of choice during 5 min. During the inter-trial intervals (2 min), the pigeon rested in a cage located in an adjacent room. After the 6th trial, the pigeons were returned to their home cages. In the first training session there were three food grains left on the top of the correct cup during the first two trials. Recording of the latency value and of accuracy of choice were further validated through the transcription of the digital recording of the sessions. Control birds were exposed to the arena during 2 (C2) or seven (C7) sessions, but the four cups were empty. Naïve birds were not exposed to the experimental situation. They underwent food deprivation and like the other birds were taken from their home-cages, transported to the laboratory, weighted and then returned to their home-cages. Control and Naïve birds were used as control for imunohistochemical analysis.

› Probe test

The test was conducted for the E2 (n=5) and E7 (n=7) birds and consisted in one session, beginning 24 hours after the last training session with three experimental trials. The first two trials were similar to the experimental trials of the previous sessions, but in the third trial the feeders were removed from the arena. The birds were then exposed to the arena during 5 min and the time spent in each of the four virtual quadrants was recorded.

» Immunohistochemistry procedures

› Perfusion

After 60 min since the end of 2nd experimental session (E2 and C2), of the 7th session (E7 and C7) or of the probe test, the birds were anesthetized with ketamine (2 mg/kg, im) and xylazine (5 mg/kg, i.m.)

and underwent cardiac perfusion with 0.9% saline and 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB, pH 7.4). The brains were post-fixed in 4% PFA during a 4-6h period and subsequently stored in a 30% sucrose solution at 4º C for at least 48h.

› Immunohistochemistry for Synapsin I protein

The brains were sectioned (30 µm) on a sliding microtome in the coronal plane. Sections were incubated with the primary antibody (rabbit polyclonal antibody against the Synapsin I protein, ab64581; abcam Cambridge, United Kingdom) – (In this research we rely on studies of Scott & Lois in 2005, using antibodies produced in rabbits to show the synapsin in birds) diluted 1:1000 in PB containing 0.3% Triton X-100. Incubation with this primary antibody lasted 12-16 h at 22°. Following three 10-min washes in PB, the sections were incubated with a biotinylated goat anti-rabbit serum (Jackson Laboratories, USA) diluted 1:200 in PB with 0.3% Triton X-100 at 22°C for 1 h. Finally, the sections were incubated for 2 h with the avidin-biotinperoxidase complex (ABC Elite Kit; Vector Labs, USA). Following, the sections were treated with 0.05% 3.3'-diaminobenzidine solution with 3 µL 0.01% H2O2 for 5 min. The sections were mounted on gelatin coated glass slides, dehydrated in an ethanol series, and over slipped with Permount (Fisher, USA). Sections from both brain hemispheres were examined under a light microscope. The immunoreactive neuronal nuclei expressed were quantitated with the Image software (NIH Image). A threshold for stained cell counting was defined on the basis of background staining, and the cells exhibiting at least three times higher absorbance than the threshold were counted. The count was reported as the density of Synapsin I protein cells (cells/mm2). A minimum of 5 sections from each subject were examined.

Statistical Analysis

ANOVA for repeated measures was used for comparisons considering group (E2, E7, C2, C7, Naïve) and session (S1-S7) as independent variables and the mean value of latency for correct choice or the mean value of accuracy index as dependent variables. Two-way ANOVA, with group (E2, E7, C2, C7, Naïve) and region of the hippocampus (DHp or VHp) as factors, was used to test he statistical differences for Synapsin I- positive cells counting. The Tukey-Kramer test was used for post hoc multiple comparisons.

Results

› Behavioral analysis of latency and accuracy of spatial food location

Figure 1A shows the schematic representation of the experimental procedure for each one of the groups (E2, E7, C2, C7, Naïve). Control groups underwent the same experimental conditions, but the feeders were empty. The mean values of latency of the correct choice response are presented for each group of pigeons trained during two or seven sessions in choice of food location (E2 and E7) and control groups (C2 and C7). The E7 birds improved their performance across sessions as indicated by values of latencies in the 4, 5, 6 and7 sessions that were lower than those seen in first three sessions (Figure 1B and 1C). The control groups did not show any feeder choice. Statistical comparisons with ANOVA confirmed significant effect of group ($F_{3,24}$ = 560,61; $p < 0.0001$), session ($F_{6,34}$ = 9,9; $p < 0.01$) and significant interaction between groups x session ($F_{3,18}$ = 27,32; $p < 0.001$). The Tukey-Kramer for multiple post hoc

comparisons indicated that the control groups differed significantly from both E2 and E7 ($p < 0.05$). The decreases in the latencies observed for sessions 4, 5, 6 and7 sessions of the group E7 were significantly different from the sessions 1, 2 and 3 ($p < 0.05$). The E7 group showed mean accuracy index values + s.e.m. (correct choice/ correct + incorrect choices) that increased across the training sessions and reached a mean value around 0.9 in the last session (Figure 1D). Analysis with ANOVA for repeated measures indicated a significant effect of group ($F=_{3,34} = 45,22$; $p < 0.001$), of session ($F=_{6,34} = 3,87$; $p < 0.05$), with significant group x session interaction ($F=_{3,18} = 2,82$; $p < 0.05$). Analyses with Tukey-Kramer test for post-hoc multiple comparisons indicated that the E7 birds accuracy index value in the last training session differed significantly from the first two sessions ($p < 0.05$) of both E7 and E2 groups ($p < 0.05$), which indicate the efficacy of the long duration training.

› Immunohistochemical Analyses of Synapsin I expression after short- and long-training

Figure 2A shows that the values of density of Synapsin I-positive cells in the hippocampus of GE7 birds was greater than the values observed for the other groups E2, C7, C2 and Naïve. The expression of Synapsin I-positive cells was higher in the dorsal region of the hippocampus (DHp) of the GE7 birds as compared to the ventral region of the hippocampus (VHp) and also to the DHp and VHp of all the groups Figure 2B The difference in number of Synapsin I-positive cells in the hippocampus of birds trained in spatial learning (GE7 and GE2), of the controls birds with non-spatial training groups (GC7 and GC2) and Naïve can be observed in the digital images (Figure 2C). ANOVA indicated a significant difference between groups ($F_{(4,20)} = 392,84$; $p < 0.0001$). The Tukey - Kramer test indicated a significant difference between both DHp and VHp of E7 with the other groups ($p < 0.05$). The VHp region of E2 birds differed significantly from E7, C7, C2 and Naïve birds ($p < 0.05$).

Discussion

This study showed proficient learning of the food location by pigeons that had long-duration training during 7 sessions, but not by pigeons receiving short-duration training during two sessions. The time to reach the food decreased and the accuracy of feeder choice increased from session to session, which confirm that the birds E7 have learned the spatial location of food. Conversely, the group E2 showed no decreases in latency and low accuracy of the choice response, which were indicative of poor learning. Furthermore, the results of the test for spatial strategy (probe test) confirmed that the pigeons E7 used spatial cues for location of the food, which was indicative of spatial memory. During this test the four feeders were removed from the arena and the pigeons E7 spent significantly longer time in the quadrant where the correct feeder was located during the training sessions whereas the pigeons E2 distributed evenly their time among the four virtual quadrants of the arena. These data may be considered indicative of spatial strategy developed during the long-duration training. Control animals that were exposed to the situation with all the empty feeders, and without any reinforcement contingencies programmed, remained still or displayed short-distance locomotion associated with visual exploration, but did not approached or choose any of the feeders during the trials. These results are according to previous avian studies showing

spatial learning in a dry-version of spatial maze by pigeons (Fremow et al., 1997), spatial learning of food location in pigeons (do Amaral-Toma & Ferrari, 2004; Kahn & Bingman, 2009), in Zebra finches (Bischoff et al. , 2006, Mayer et al., 2010, Mayer, Watanabe, & Bischof, 2013).

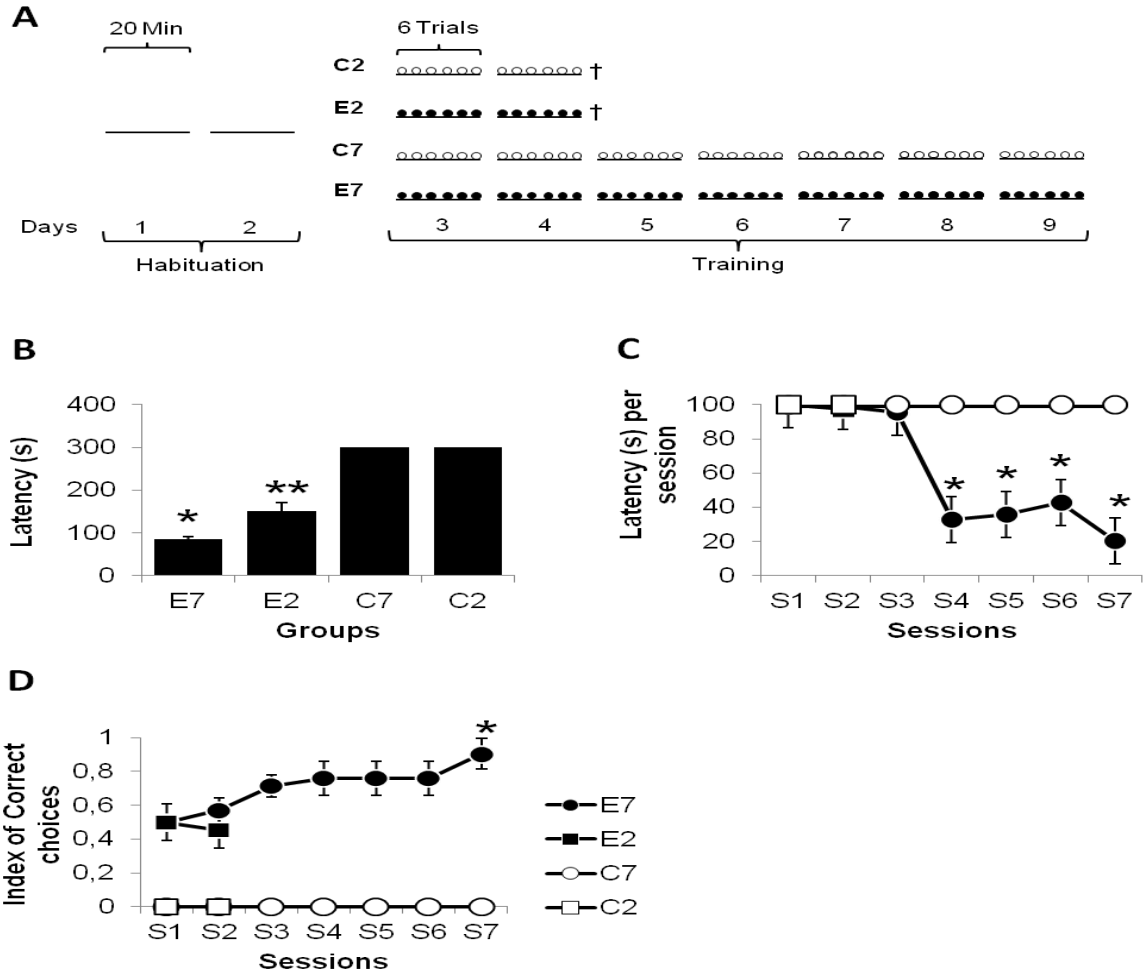

Figure1. **(A)** Schematic representation of behavioral procedures. The pigeons in the E2, E7, C2 and C7 groups were submitted to spatial memory training during 2 or 7 sessions with 6 trials per day. White circles represent empty feeders, black circles represent feeder with food. **(B)** Overall mean latency of response of correct food choice in different groups: E2 (Experimental 2 days of training), E7 (Experimental 7 days of training), C2 (Control 2 days of training), and C7 (Control 7 days of training). One way ANOVA analysis indicated significant differences between groups. Data are presented as mean ± SEM for n = 7 pigeons per group * Significant difference compared to the other groups ($p < 0.05$), ** Significant difference in relation to the other groups ($p < 0.001$) (One-way ANOVA test followed by Tukey-Kramer test for multiple comparisons). **(C)** Mean latency of the response of choice across training sessions. * Significant difference compared to sessions 1, 2 and 3 ($p < 0,05$) (Two-way ANOVA test followed by Tukey-Kramer test for multiple comparisons). **(D)** Mean values of accuracy index (correct choice/ correct + incorrect choices) for each of the seven training sessions. Each point represents data pooled for six trials/session. Data are presented as mean ± SEM for n = 7 pigeons per group * Significant difference compared sessions 1 and 2 ($P < 0.05$) (Two-way ANOVA test followed by Tukey-Kramer test for multiple comparisons).

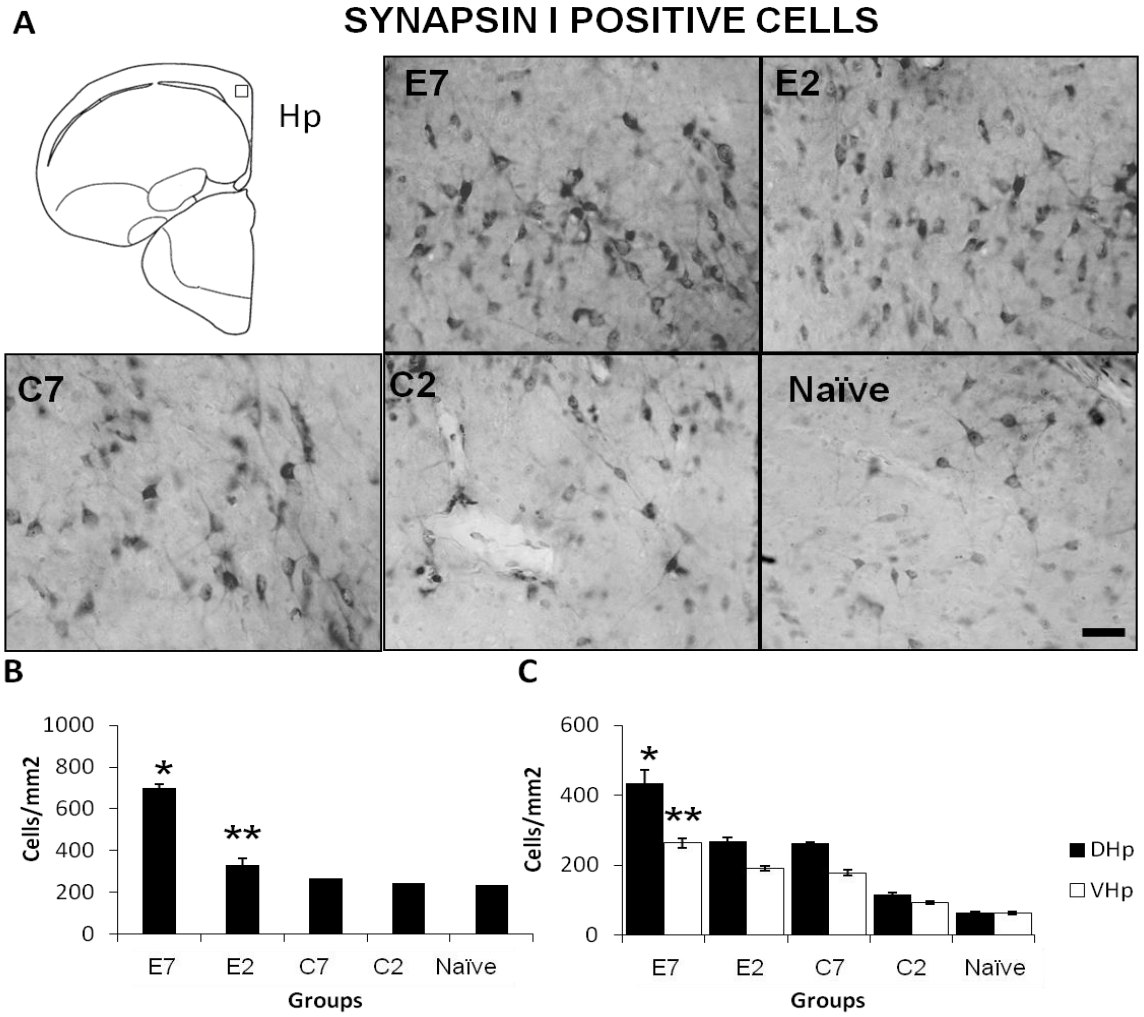

Figure 2. **(A)** Schematic representation of a frontal section of the pigoen's brain (adapted from Karten & Hodos, 1967). Digital images obtained from the sections of the dorsal hippocampus of E7, E2, C7, C2 and Naïve birds, showing Synapsin-positive cells immunolabelling. **(B)** Mean values of density of Synapsin-positive cells in the total hippocampus (DHp + VHP) in E7, E2, C7, C2 and Naïve birds. * significant difference compared to the other groups ($p < 0.05$); ** significant difference groups compared to E2 , C7, C2 and Naïve ($p < 0.05$) (One-way ANOVA test followed by Tukey-Kramer test for multiple comparisons). **(C)** Mean density of Synapsin-positive cells in the (DHp - dorsal hippocampus and VHp - ventral hippocampus) of E7, E2, C7, GC2 and Naïve. * Significant difference compared to the other groups ($p < 0.05$). ****** Significantly different when compared to DHp and VHp areas ($p < 0.05$). **Scale bar** = 200 mm

Our study revealed also that Synapsin I immunoreactions had a prominent increase in the hippocampus of E7 birds. Synapsin I is part of a phosphoprotein family involved in neuronal regulation of neurotransmitter release, with activity regulated by phosphorylation of *CamKII* and is closely related to plasticity changes. *CREB* and *CamKII* are regulated and activated by Ca2 influx mediated by *N*-methyl-D-aspartate (NMDA) receptor and Ca2_/calmodulin. The data on increased immunoreactivity of

Synapsin I following training in spatial food location, are consistent with many studies on learning and memory. Chau, Davis y Galvez (2013) reported that five days of associative learning in adults rats increased Synapsin I expression in layer IV of primary somatosensory cortex. Physical exercise has been shown to promote changes in the activational stage of Synapsin I (Szabo, Ying, Radak & Gomez-Pinilla, 2010). Additionally, synaptic activity that activates the Synapsin I cascade is critical for spatial learning. Studies have shown that hippocampal dependent learning stimulates the BDNF (Gómez-Pinilla et al., 2001) and ERK ½ (Kushner et al., 2005) dependent phosphorilation of Synapsin I and that deficits in spatial learning were related with low level of Synapsin I (Corradi et al., 2008). Synapsin I also plays a role in olfactory associative learning in *Drosophila* (Michels et al., 2005). However, Rapanelli, Frick and Zanutto (2009) found that the expression of *Synapsin I* in the hippocampus of rats was higher during initial learning of an operant task than once the task was learned. This difference between the groups was interpreted as reflecting a decrease of synaptic plasticity in the group that completed the training. Although increments of *Synapsin I* mRNA in an instrumental learning haven been related with synaptic function and with increased plasticity (Gomez-Pinilla et al.). We still need additional investigations for a better understanding on these issues.

In the present study, the cellular analysis was conducted considering the subdivision of the hippocampus in dorsal and ventral regions (Kahn et al., 2003). Both dorsal and ventral areas correspond to subdivisions of the hippocampus that are characterized by a complex neurochemical profile already described by Erichsen et al. (1991). Recent studies presented some important internal connections supporting the proposal of an avian hodological organization that could be similar to mammalian three-synaptic circuit (Hough, Pang, & Bingman, 2002; Kahn et al. 2003). In the pigeon, the sensorial information arrives through the dorsal area, specifically into the dorsolateral area with projections to the dorsomedial area. Then, a double circuit flows to ipsolateral and contralateral ventral “V” arm and lateral “V” arm. After this point, the circuit runs across the dorsal area to other pallidal areas (Atoji & Wild, 2006). The differences in labelling of Synapsin I which were found in DHp and in VHp can be seen as evidence of a differential distribution across the hippocampal regions, and regional recruitment of neuronal activity mediated by Synapsin I within the hippocampus in a situation requiring spatial memory. Regional activation of the hippocampus was also reported by other studies measured expression of immediate early gene with rats and pigeons trained in classical aversive conditioning (Hall, Thomas, & Everitt, 2001).

In conclusion, our present results confirmed a role for the activation of Synapsin I in neuronal circuits of the hippocampus in pigeons during the formation and the persistence of spatial memory of food location in pigeons. The data point to the need for additional studies to better clarify the role played by these proteins within the highly complex and dynamic molecular network that is activated during the different phases of spatial memory formation and persistence and the underlying plasticity. Additionally, is possible to say that our data contribute and stimulate discussions on the many behavioral, neural and molecular mechanisms that are conservative in the avian and mammalian hippocampus.

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