UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

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RELACIÓN DE LA ACTIVIDAD OSCILATORIA THETA DE LA VÍA SEPTO-HIPOCAMPAL Y SEPTO-MAMILAR DURANTE EL APRENDIZAJE ESPACIAL EN RATAS CON ALTERACIÓN SEROTONINÉRGICA SEPTAL

TESIS

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ABREVIATURAS

SL Septo lateral

SM Septo medial

BD Banda diagonal de broca

BDv Brazo vertical de la banda diagonal de Broca

BDh Brazo horizontal de la banda diagonal de Broca

SM/BDBvh Septo medial / banda diagonal de Broca. Para simplificar, en el texto y figuras de la tesis será referido solo como SM.

SUM Núcleo supramamilar

MM Núcleo mamilar medial de los cuerpos mamilares

Área mamilar Incluye al SUM y MM

RPO Núcleo reticular de pontis oralis

MFC haz medial frontocerebral

RM Rafé medial

LAM Laberinto acuático de Morris

EEM Error estándar de la media

5,7-DHT Neurotóxico 5,7-dihidroxi-triptamina

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RESUMEN

La actividad theta hipocampal ha sido relacionada con el procesamiento de información espacial y la formación de la memoria dependiente del hipocampo. El septo medial (SM) juega un papel importante en el control y coordinación de la actividad theta, así como en la modulación del aprendizaje. La lesión o inactivación del SM interrumpe la actividad theta y causa deficiencias en el aprendizaje de una tarea de memoria de referencia. Neuronas serotoninérgicas del rafé inervan principalmente al hipocampo y al SM. Un incremento de la actividad serotoninérgica puede desincronizar la actividad theta, mientras que una actividad serotoninérgica reducida produce actividad theta continua y persistente en el hipocampo. Además, se ha propuesto que un bajo tono serotoninérgico en el SM podría facilitar la codificación de información. Se ha sugerido que la serotonina puede modular el aprendizaje a través de la modulación en la sincronía hipocampal. En este estudio, se investigó si la serotonina en el SM podría modificar el aprendizaje espacial y la relación funcional de la actividad theta septo-hipocampal y septo-mamilar. A un grupo de ratas de la cepa Sprague-Dawley se le redujo la serotonina del septo medial (5HT-D) mediante la microinyección del neurotóxico 5,7-dihidroxitriptamina sobre SM. Se registró la actividad theta en el hipocampo, el SM, los núcleos supramamilar y mamilar medial durante el aprendizaje espacial evaluado en el laberinto acuático de Morris. A otro grupo de ratas (CITAL) se les indujo un aumento de la serotonina en el SM mediante la aplicación intraseptal del inhibidor de la recaptura de serotonina citalopram y se evaluó el aprendizaje espacial. En el grupo 5HT-D, se observó una facilitación en el aprendizaje espacial y se asoció con un incremento de la frecuencia de la actividad theta hipocampal (a 8.5 Hz) durante los primeros días de entrenamiento con respecto al grupo vehículo. Además, fue mayor la coherencia entre SM-hipocampo y SM-núcleos mamilares durante el segundo día de prueba, con respeto al grupo vehículo. Se demostró que la reducción de serotonina septal facilita la adquisición de información espacial en asociación con un mayor acoplamiento funcional del SM con el hipocampo y los otros núcleos. Por otro lado, en el grupo CITAL se observó una menor eficiencia para resolver la tarea de aprendizaje espacial. Esto apoya la hipótesis de que un bajo tono serotoninérgico en el SM facilita la adquisición de información, mientras que un alto tono de serotonina afecta la codificación de información, y esto puede estar asociado con el papel de la serotonina como desincronizador de la actividad theta para facilitar o impedir la codificación de información a través del SM. La serotonina que actúa en el septo medial modula la actividad theta hipocampal y el aprendizaje espacial.

ABSTRACT

Hippocampal theta activity has been related to the processing of spatial information and the formation of hippocampus-dependent memory. The medial septum (MS) plays an important role in the control and coordination of theta activity, as well as in the modulation of learning. Lesions or inactivation of the MS disrupts hippocampal theta activity and causes learning deficits in reference memory task. Serotonergic raphe neurons prominently innervate the hippocampus and MS. An increased serotonergic activity may desynchronize theta activity, while reduced serotonergic activity produces continuous and persistent theta activity in the hippocampus. It has been proposed that serotonin may regulate learning through the modulation of hippocampal synchrony. We investigate whether serotonin acting on the MS could modify spatial learning and the functional relationship between septo-hippocampal and septo-mammillary theta activity. The serotonin was depleted from the medial septum (5HT-D) by the injection of neurotoxic 5,7- dihydroxytryptamine in MS. Theta activity was recorded in the hippocampus, medial septum, supramammillary and medial mammillary nuclei of Sprague-Dawley male rats during the performance of spatial learning evaluated in the Morris water maze. Another group (CITAL), was increased MS serotonin by application of intra-septal citalopram and spatial learning was evaluated. In the 5HT-D group, spatial learning was facilitated, and the frequency of the hippocampal theta activity during the first days of training increased (to 8.5 Hz) in the 5HT-D group, unlike the vehicle group. Additionally, the coherence between the MS-hippocampus and the MS-mammillary nuclei was higher during the second day of the test compared to the vehicle group. We demonstrated that septal serotonin depletion facilitates the acquisition of spatial information in association with a higher functional coupling of the medial septum with the hippocampus and mammillary nuclei. On the other hand, in the CITAL group, a lower efficiency was observed to solve the spatial learning task. This supports the hypothesis that a low serotonergic tone to MS facilitates the acquisition of information, whereas a high tone of serotonin affects the encoding of information, and this may be associated with the idea of serotonin as the desynchronizer of theta activity to facilitate or prevent the encoding of information through the MS. Serotonin, acting in the medial septum, modulates hippocampal theta activity and spatial learning.

1. INTRODUCCIÓN

La actividad theta del hipocampo es importante para el procesamiento de información relacionado con el aprendizaje y la formación de la memoria, además, una actividad rítmica y sincrónica de la actividad theta puede facilitar la codificación de información y ser necesaria para su almacenamiento y recuperación. Se ha propuesto que el sistema serotoninérgico originado en el rafé medial actúa como un desincronizador de la actividad theta del hipocampo. Esta hipótesis se ha derivado de estudios en ratas, en los cuales se muestra que la estimulación a alta frecuencia del rafé medial, o un incremento de la actividad serotoninérgica produce la pérdida de la actividad rítmica theta del hipocampo, mientras que una supresión de la actividad serotoninérgica produce una actividad theta continua y persistente.

Un punto interesante es que la mayoría de los estudios con respecto a la serotonina como desincronizador y modulador de la actividad theta hipocampal, han sido realizados principalmente en animales anestesiados o con libertad de movimiento en su caja y a partir de ellos, se han planteado hipótesis acerca de la implicación funcional que podría tener un estado de alto o bajo tono serotoninérgico hacia el hipocampo y áreas relacionadas, como el septo medial. Las hipótesis propuestas sugieren que la información que llega al hipocampo durante la ocurrencia de la actividad theta (estado sincronizado, por baja actividad serotoninérgica), seria guardada o codificada por lo menos temporalmente en el hipocampo, mientras que aquella información que llega en ausencia de actividad theta (estado desincronizado, ocasionada por alta actividad serotoninérgica), no sería codificada (Vertes y Kocsis, 1997). Por lo tanto, esto podría ser un mecanismo para ignorar eventos ambientales no significativos, para la selectividad de codificación de la información relevante (Vertes, 2005), además, algunos autores sugieren que el incrementar el tono serotoninérgico (desincronización de la actividad theta) hacia el septo medial, se impide la liberación de acetilcolina y glutamato hacia el hipocampo, afectando la codificación de información y por lo tanto impide el aprendizaje; mientras que un bajo tono serotoninérgico (actividad theta sincronizada) hacia el septo medial, facilitaría la liberación de acetilcolina y glutamato hacia el hipocampo,

favoreciendo la codificación de información y el aprendizaje (Jeltsch-David et al., 2008).

Sin embargo, estas hipótesis acerca del papel funcional de la serotonina sobre la actividad theta durante el aprendizaje no han sido confirmadas. Es decir, no se sabe si lo que se ha reportado en animales anestesiados ocurre también durante conductas que impliquen la codificación y recuperación de la información, como en una tarea de aprendizaje y memoria que depende de la función del hipocampo.

Para probar el papel funcional de la serotonina sobre la actividad theta, se pretende modificar la serotonina del septo medial (marcapaso de la actividad theta) y evaluar su repercusión sobre la actividad theta durante el aprendizaje dependiente del hipocampo. Una modificación de la serotonina en la región septal podría ocasionar un desbalance en la actividad neuroquímica del circuito intra-septal, generando una repercusión funcional sobre la fisiología del hipocampo y del área mamilar, ésta última es importante en la determinación de la frecuencia de la actividad theta y el aprendizaje. Si la serotonina desincroniza la actividad theta durante el aprendizaje, sería de esperarse que un incremento de la actividad serotoninérgica del sitio que controla y determina la actividad theta (septo medial), reflejara una disminución de la actividad theta, en asociación con probables deficiencias en el aprendizaje. Mientras que una reducción de la actividad serotoninérgica podría ocasionar predominancia de la actividad theta asociada a una mayor eficiencia en el aprendizaje. Dada la importancia del septo medial en la coordinación temporal del flujo de información hacia el hipocampo y área mamilar (mediante los circuitos septo-hipocampal y septo-mamilar), y por lo tanto en el control y determinación de la actividad theta, se sugiere que la desincronización y sincronización de la actividad theta hipocampal ocasionada por cambios en la actividad serotoninérgica, puede deberse a la modificación de las propiedades del septo medial que a su vez influirán sobre la función del hipocampo y la región mamilar, ya que ambos estados (desincronización/sincronización) nos estarían indicando diferentes modos del procesamiento de la información.

2. ANTECEDENTES

2.1 ACTIVIDAD THETA HIPOCAMPAL

La actividad theta hipocampal se presenta principalmente durante conductas que implican la extracción de información ambiental, como la actividad exploratoria, locomoción, nado, olfateo, movimientos de cabeza y durante el sueño MOR (Buzsaki, 2005; Kahana, Seelig, y Madsen, 2001; Vandewolf, 1969). Además, predomina durante conductas asociadas a la adquisición de información para la formación de la memoria y el aprendizaje (Winson, 1978). También puede aparecer espontáneamente o puede ser generada por varios estímulos sensoriales y por activación eléctrica o farmacológica de la formación reticular del tallo del cerebro (Vertes, 1981).

Las oscilaciones theta son fluctuaciones del potencial de campo local, registrables en varias partes del cerebro, pero son predominantes en el hipocampo. Se caracterizan por presentar un patrón de actividad rítmica lenta sinusoidal con una amplitud de 1 a 2 mV y una frecuencia de 4 -12 Hz en ratas y de 4-7 Hz en humanos. A pesar de que la actividad intrínseca de circuito intrahipocampal puede generar actividad theta, esta puede ser modificada por la entrada de información originada en regiones extrahipocampales (Vanderwolf, 1988) que permiten sincronizar tales oscilaciones (Vertes y Kocsis, 1997). Las oscilaciones a frecuencia theta pueden ser generadas por la entrada de corrientes negativas y positivas hacia las células principales del hipocampo (glutamatérgicas) formando fuentes y sumideros, que ocasionan fluctuaciones de corriente extracelular (Buzsaki, 2002).

Cuando se realizan registros a varias profundidades por los diferentes estratos del hipocampo, se puede observar la presencia de actividad theta con una máxima amplitud en la zona del estrato oriens-piramidal del área de CA1 y en el estrato molecular de la hoja dorsal del giro dentado, pero con un desfasamiento de aproximadamente 180°, con respecto a la actividad theta de CA1 (Monmaur y Thomson, 1986; Bland y Whishaw, 1976; Buszaki, 2002). Se ha propuesto un modelo de la generación de la actividad theta, en el cual del septo medial (a través de las células GABAérgicas) juega un papel muy importante para la sincronización

de esta actividad, ya que es fuente de la principal entrada de información subcortical hacia el hipocampo y actúa como un sitio de relevo de información proveniente del tallo cerebral e hipotálamo, lo que se describirá más adelante, en la sección del septo medial.

La actividad theta es importante en el hipocampo para el procesamiento y registro de información (Vinogradova et al., 1993; Bland y Colom, 1993; Vertes y Kocsis, 1997), por ejemplo, se le ha relacionado con el aprendizaje y navegación espacial (Buzsáki, 2005), pues participa en la codificación de la posición de los individuos en el espacio (O´Keefe y Recce, 1993). Se ha mostrado que ocurre un aumento de la potencia de la banda theta de alta frecuencia durante la realización de una prueba en el laberinto acuático de Morris que implica aprendizaje de lugar y no durante el aprendizaje de señal que no depende de la función del hipocampo (Olvera-Cortes et al., 2004). Así, también se ha mostrado que deficiencias en el aprendizaje espacial están asociadas con una disminución de la actividad theta hipocampal (Winson, 1978). Por otro lado, se ha visto que la actividad oscilatoria en frecuencias theta predomina durante tareas de memoria de corto plazo (Vertes, 2005). Por lo que se ha sugerido que refleja “en línea” los estados del hipocampo; como una preparación para procesar señales en curso (Buzsáki, 2002). En los seres humanos se han observado episodios de actividad theta en el EEG durante la navegación virtual (Nishiyama, Mizuhara, Miwakeichi y Yamaguchi, 2002), así como un incremento en la actividad theta cuando hay aumento en el aprendizaje y la memoria espacial (Kahana, 2001).

Como ya se ha mencionado, la actividad theta del hipocampo es controlada por la entrada de información subcortical, que asciende del tallo del cerebro con relevo en el área septal, y a través de sus conexiones reciprocas del septo con el hipocampo forman el circuito septo-hipocampal, determinante para la función del hipocampo (Vertes y Koscsis, 1997).

2.2 SISTEMA SEPTO-HIPOCAMPAL

2.2.1 Hipocampo

En los mamíferos el hipocampo se extiende dorsalmente desde el septum hasta la parte caudal de la amígdala ventral (Noback y Demarest, 1975). Es una estructura enrollada sobre sí misma en los dos extremos, en forma de C. El hipocampo ha sido dividido en una parte dorsal o septal y una parte ventral o temporal (Amaral y Witter, 1989; Johnston y Amaral, 1998). En un corte perpendicular al eje septotemporal (eje largo) del hipocampo se observan varios campos de la formación hipocampal y varias de las conexiones intrínsecas (Figura 1).

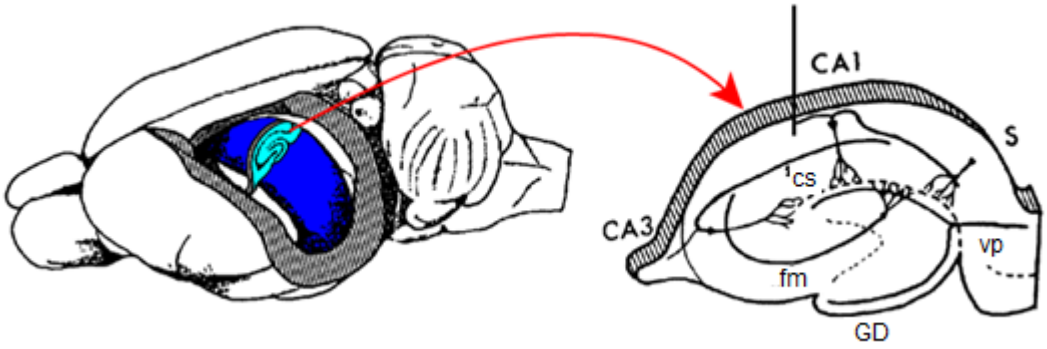


Figura 1. Representación esquemática de la localización del hipocampo en el cerebro de la rata. Modificada de Eric Hargreaves'PageO'Neuroplasticity: homepages.nyu.edu/~eh597/seahorse.htm. fm, fibras musgosas; vp, via perforante; cs, colaterales de Schaffer; S, subiculum; GD, giro dentado.

El hipocampo está formado por el cuerno de Ammon (*cornu Ammonis*, CA) que incluye a tres principales regiones CA1, CA2 y CA3, distinguibles por el tamaño y apariencia de sus neuronas, por su patrón de conexiones y por su posición con respecto al giro dentado (Amaral y Witter, 1995). La capa de células principales está formada por células piramidales (multipolares) de carácter glutamatérgico, y a su vez se divide en diferentes estratos según el arreglo de las células. Al iniciar por la zona cercana a la superficie ventricular, se encuentra al *alveus,* como una hoja delgada de fibras entrantes y de salida*;* enseguida se localiza el *estrato oriens* que está ocupado por dendritas basales de las células piramidales y se continua con el

estrato piramidal que está constituido por los cuerpos de las células piramidales; el *estrato radiado* queda superficial a la capa de células piramidales, y finalmente, en el *estrato lacunoso-molecular* se localizan las dendritas apicales de las células piramidales (Amaral y Witter, 1995). Figura 2

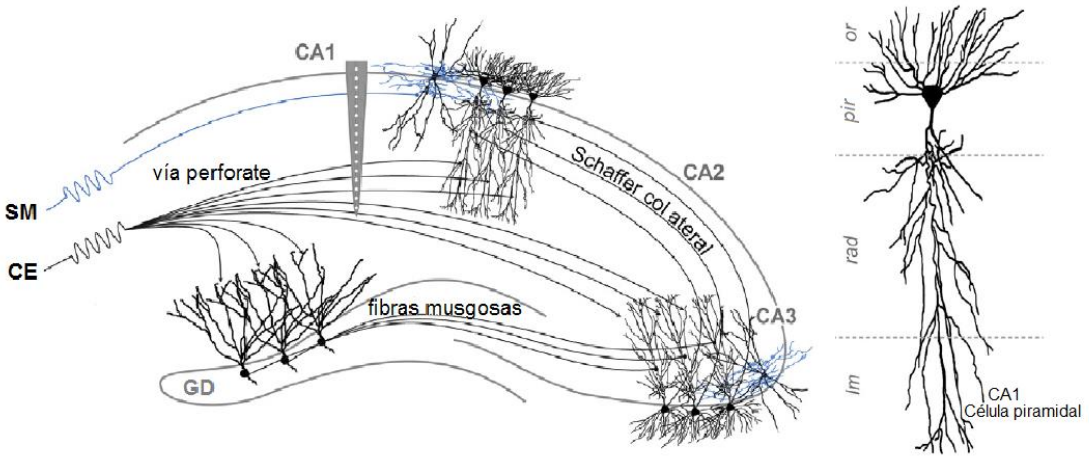


Figura 2. Circuito hipocampal que muestra la entrada de corrientes excitadoras e inhibitorias del SM Y CE hacia las diferentes regiones y estratos del hipocampo. MS, septo medial; CE, corteza entorrinal; estratos: or, oriens; pir, piramidal; rad, radiado; lm, lacunoso molecular. Modificado de Pignatelli, et al 2011.

En el hipocampo existen interneuronas de naturaleza GABAérgica de diferentes tipos (todas inhibitorias) como parte de los circuitos locales, particularmente las interneuronas con inmunoreactividad positiva a calbindina envían proyecciones hacia el septo medial (Freund y Buzsaki, 1996). Las interneuronas de CA1 reciben conexiones sinápticas de células noradrenérgicas y serotoninérgicas (Ropert, Miles y Korn, 1990). El giro dentado consiste en tres capas: la capa de células granulares (principalmente monopolares), una gran capa molecular acelular localizada sobre la capa de células granulares y una capa difusa de células polimórficas (algunos la llaman región hilar o hilus) que se localiza debajo de la capa de células granulares. Todas las células son de naturaleza glutamatérgica. También existen tipos de interneuronas análogos a los de CA1, y están localizadas entre la capa de células granulares y la capa de células polimórficas (Johnston y Amaral 1998).

Como se muestra en las Figuras 2 y 3, en el circuito básico del hipocampo, la corteza entorrinal (CE) es la principal entrada de información al hipocampo, envía información a las células granulares del giro dentado (GD) a través de la vía perforante (glutamatérgica) y a las células piramidales de CA3. A su vez las células del GD hacen sinapsis con las células de la región de CA3 por medio de las fibras musgosas que envían información a la región de CA1 a través de las colaterales de Schaffer. La región de CA1 recibe aferentes de la CE como parte de la vía directa (monosináptica). Esta región a su vez proyecta a la CE y al subiculum, siendo esta la vía de salida de información procesada en el hipocampo (Johnston y Amaral, 1998).

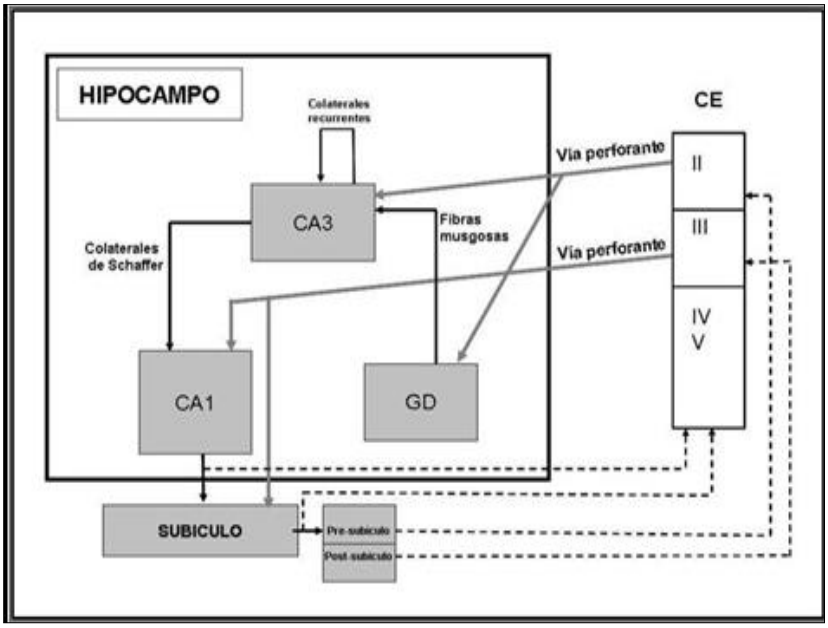


Figura 3. Representación esquemática del circuito del hipocampo. CA1, CA3, regiones del Cuerno de Ammón; GD, giro dentado; CE, corteza entorrinal; II, III, IV, y V, capas de la corteza entorrinal. Modificada de Gutiérrez-Guzmán, et al 2007.

Existen evidencias que indican que el hipocampo participa en la aprendizaje y memoria espacial, en el aprendizaje relacional, en la memoria episódica y en la memoria declarativa (O`Keefe y Nadel, 1978; Eichenbaum, 1999; Tulving, 2002; Squirre, Stark y Clark, 2004; Moscovitch et al., 2005).

En estudios clínicos de pacientes con lesiones en el lóbulo temporal (un ejemplo de ellos es el paciente HM) se observó que pierden la habilidad para generar nueva

memoria a largo plazo para eventos nuevos, es decir presentan amnesia anterograda (Corkin et al., 1997; Milner y Penfield 1955). También se ha demostrado un papel esencial del hipocampo en la formación de la memoria explicita o declarativa, pero no en la implícita, ya que los pacientes no pueden recordar nuevas caras y lugares, pero pueden aprender nuevas destrezas motoras (Milner, Squire y Kandel, 1998).

Diferentes autores sugieren que el hipocampo no es el almacén de la memoria en el largo plazo, si no que ayuda a procesar información para ser almacenada en el cerebro a largo plazo (Kim y Fanselow, 1992; Winocur, 1990). De tal manera que funciona como un almacén de memoria temporal (Rawlins, 1985) o como un almacén de plazo intermedio (Treves y Rolls, 1994).

Por otro lado, hay estudios experimentales que indican que las lesiones del hipocampo o de las estructuras asociadas (fimbria fórnix, septum, corteza entorrinal y complejo subicular) provocan deficiencias graves y permanentes en numerosas habilidades espaciales como la navegación en un ambiente que demanda la asociación de las señales que se encuentran en él, para llegar a una meta, es decir en el aprendizaje y la memoria espacial (Morris, et al., 1990; O´keefe y Nadel, 1978). En relación con esto, O'Keefe y Dostrovsky (1971) descubrieron que existen neuronas del hipocampo a las cuales se ha nombrado "células de lugar", que se activan específicamente cuando el animal está en una localización determinada del ambiente, forman un mapa cognitivo en el hipocampo y se les ha considerado como elementos de una representación cartesiana del ambiente (Nadel y Eichenbaum, 1999). Anatómicamente son células piramidales de la región CA1 y CA3 (Muller, 1996).

Entre otras teorías acerca de la participación del hipocampo en el aprendizaje y la memoria, Rudy y Suterland (1995) sugirieron la teoría de la asociación configural en la que el sistema hipocampal combina la representación de eventos para formar representaciones únicas. Por su parte, Eichenbaum (1999) propuso una teoría relacional, en la que la representación del espacio se desarrolla mediante una amplia colección de codificaciones en las que se superponen rasgos determinados de las representaciones episódicas, determinando y restringiendo la representación

global de las relaciones espaciales entre las señales del ambiente (pistas). Se basa en el procesamiento de estímulos visuales, estableciendo que el hipocampo no es necesario para codificar ni expresar memorias independientes, pero si resulta crucial en la codificación y expresión de memorias que requieren relaciones entre eventos o unidades de información, tanto temporales como configuracionales (Eichenbaum, 1999; Eichenbaum y Cohen, 2001).

La función del hipocampo también es controlada por una de las principales vías de información subcortical, el septo medial, que es un sitio de relevo de información muy importante para el establecimiento del aprendizaje y la memoria espacial, así como la actividad theta.

2.2.2 Región septal

La región septal es una parte del sistema límbico del cerebro, localizado entre el cuerno anterior del ventrículo lateral (septum=*saeptum* en latín; una pared divisoria o membrana), y la parte dorsal de la línea media de la comisura anterior, también se localiza ventral a la región media y anterior del cuerpo calloso. Puede ser considerado como una interfase entre el diencéfalo y telencéfalo con conexiones masivas y recíprocas. De acuerdo con Jakab y Leranth, la región septal puede ser dividida en tres partes, el septum lateral (SL), septo medial / banda diagonal de Broca, y septo posterior (Jakab y Leranth, 1995).

El complejo septo medial / banda diagonal de Broca consiste en el núcleo septal medial (SM) y la banda diagonal de broca (BD), esta última incluye dos partes, el brazo vertical de la banda diagonal de Broca (BDv) y el brazo horizontal de la banda diagonal de Broca (BDh). No son claros los límites anatómicos entre el SM y la BDv, ya que hay similitudes neuroquímicas y funcionales entre ellos. Para facilitar la lectura, este complejo septal será referido en el texto de la presente tesis como SM. El SL, está dividido en tres partes: la dorsal, intermedia y ventral (Jakab y Leranth, 1995).

El papel funcional del SM y SL son diferentes, mientras el SM releva e integra información ascendente del diencéfalo y telencéfalo, el papel del SL es

principalmente mediar información descendente del telencéfalo al diencéfalo (Jakab y Leranth, 1995).

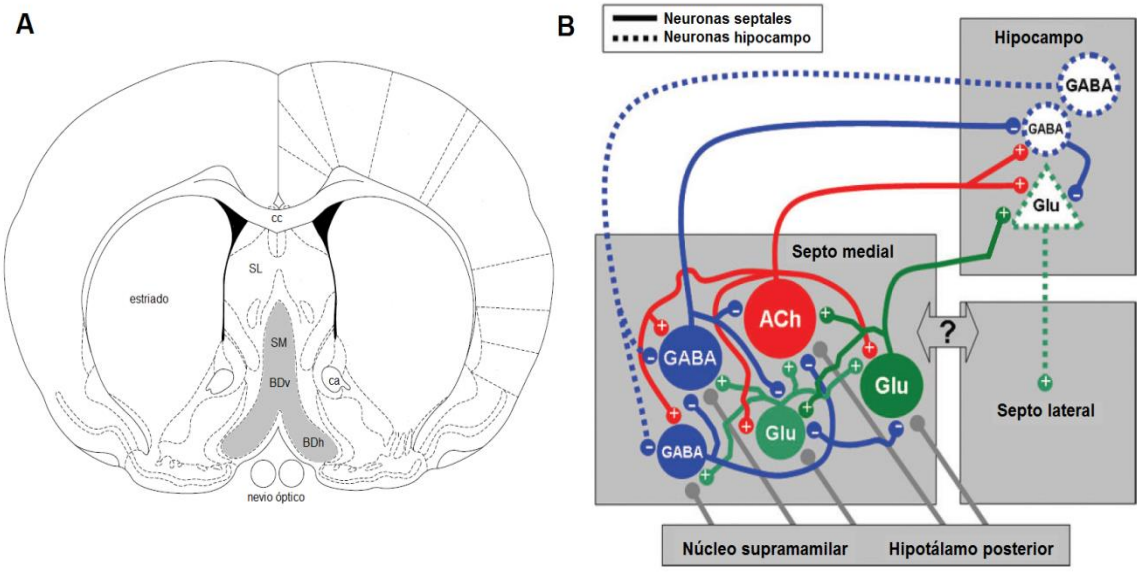


Figura 4. (A) Sección coronal del septo medial y (B) su conexión con el hipocampo. Neuronas colinérgicas (Ach), GABAérgicas (GABA) y glutamatérgicas (Glu) hacen sinapsis con células del hipocampo. Entrada de información ascendente de núcleo supramamilar e hipotálamo posterior hacia el SM. SM-septo medial, BDv-brazo vertical de la banda diagonal de Broca, BDh-brazo horizontal de la banda diagonal de Broca, SL-septo lateral, cc-cuerpo calloso. Modificado de Paxinos y Watson, 1998; Colom, 2006.

El SM integra y releva información originada del tallo del cerebro, del núcleo supramamilar, del núcleo del hipotálamo posterior y de la corteza entorrinal, la cual es transmitida hacia el hipocampo por aferentes colinérgicas, GABAérgicas y glutamatérgicas (Jaskiw et al., 1991; Leranth et al., 1999; Leranth y Kiss, 1996). Las células del SM proyectan primariamente al hipocampo a través de un arreglo topográfico de manera medio-lateral. Las células del SM localizadas lateralmente proyectan a la parte ventral-lateral del hipocampo y a la porción medial de la corteza entorrinal, mientras que las células de la parte más medial inervan a la parte septal-dorsal del hipocampo y a la parte más lateral de la corteza entorrinal (Gaykema et al., 1990).

Dentro del circuito interno del SM, axones colaterales colinérgicos terminan sobre células GABAérgicas y glutamatérgicas; las colaterales de neuronas GABAérgicas

terminan sobre las células colinérgicas de la BD y otras neuronas GABAérgicas (Alreja et al., 2000; Brashear et al., 1986; Kiss et al., 1990); las neuronas glutamatérgicas terminan sobre las neuronas colinérgicas y GABAérgicas (Manseau et al, 2005; Huh et al, 2010).

En el circuito septo-hipocampal, existen conexiones reciprocas. Las neuronas colinérgicas hacen sinapsis con células piramidales principales e interneuronas del hipocampo (Frotscher y Leranth, 1985). En contraste los axones de las células GABAérgicas terminan exclusivamente sobre interneuronas GABAérgicas del hipocampo, lo cual permite una desinhibición de las células piramidales (Freund y Antal, 1988; Toth et al., 1997). Neuronas glutamatérgicas septales hacen sinapsis con células piramidales del hipocampo (Sotty et al, 2003; Huh, et al., 2010). El hipocampo envía aferentes descendentes al SM y al SL. Una porción de neuronas GABAérgicas del hipocampo envían axones de proyección que terminan sobre las células GABAérgicas del SM (Jacab y Leranth, 1995; Alreja et al., 2000), mientras que neuronas glutamatérgicas del hipocampo tienen conexión con neuronas GABAérgicas del SL (Jacab y Leranth, 1995).

Las células septales están organizadas en un patrón laminado, neuronas GABAérgicas que contienen parvalbumina se localizan predominantemente en la zona media y las colinérgicas en la zona lateral de SM (Jakab y Leranth, 1995; Luttgen et al., 2005).

En los circuitos ya mencionados, el septo medial tiene un papel muy importante para la función hipocampal. Particularmente sobre los procesos de aprendizaje y memoria espacial y la actividad theta.

2.2.2.1 Relación funcional del septo medial con el hipocampo

El SM como parte del sistema de sincronización ascendente es una estación de relevo de información en el circuito que conecta con el hipocampo y regiones límbicas subcorticales, como los núcleos supramamilar y mamilar medial (Jakab y Leranth, 1995; Pan y McNaughton, 2004).

El SM contiene células que disparan rítmicamente y están directamente involucradas en la generación del ritmo theta hipocampal (Leão et al, 2015). Se ha

propuesto que el SM funciona como un “marcapaso” que encarrila la formación de la actividad theta en el hipocampo (Bland, 1986; Vertes, 1986; Stewart y Fox, 1990). Es el encargado de convertir el flujo constante no-rítmico de pulsos de la formación reticular, en actividad theta rítmica (Petsche et al, 1965), es decir participa en la coordinación temporal del flujo de información hacia el hipocampo (Andersen et al, 1979; Kirk y McNaughton, 1991; Wang, 2002). Esta propuesta ha sido apoyada por estudios en que se muestra que la lesión o inhibición con lidocaína del SM elimina completamente la actividad theta del hipocampo y la corteza entorrinal (Andersen et al., 1979; Leung et al, 1994; Lawson y Bland, 1993).

También, en estudios farmacológicos se ha mostrado que las células colinérgicas del SM regulan la actividad theta hipocampal, ya que se ha encontrado que la aplicación de agonistas colinérgicos (carbacol) de forma sistémica y mediante la microinfusión dentro del septum (Monmaur y Breton, 1991) o del hipocampo (Colom et al, 1991) producen actividad theta. Por el contrario, los antagonistas colinérgicos atenúan la actividad theta (Bennett et al, 1971; Kramis et al, 1975) y la lesión colinérgica septal mediante la infusión de 192-IgG-saporina, reduce la actividad theta en el hipocampo (Lee et al, 1994). Además, antagonistas muscarínicos también atenúan la actividad theta en asociación con el deterioro de la memoria espacial (Bennett et al, 1971). Por otro lado, se ha demostrado que las neuronas GABAérgicas también modulan la actividad theta hipocampal (Yoder y Pang, 2005), ya que la lesión selectiva de células GABAérgicas reduce la potencia de la actividad theta y resulta en la pérdida del disparo rítmico de neuronas septo-hipocampales (Hangya et al, 2009; Sotty et al, 2003).

En varios estudios se ha mostrado que la inactivación o lesión del SM en roedores, induce severas deficiencias en el aprendizaje y memoria espacial evaluado en el laberinto acuático de Morris (Kelsey y Landry, 1988; Leutgeb y Mizumori, 1999; Winson, 1978). Además, la lesión colinérgica en el SM ocasiona deficiencias en la memoria espacial (Bennett et al, 1971; Craig et al, 2009), sin embargo, también se ha mostrado que después del daño selectivo de las neuronas colinérgicas septales, las ratas son capaces de adquirir la localización de la plataforma en la tarea del laberinto acuático de Morris (Berger-Sweeney et al, 1994; Baxter et al, 1995).

También se ha demostrado la participación de neuronas GABAérgicas septo-hipocampales en la adquisición y consolidación de la memoria espacial (Lecourtier et al, 2011) ya que la lesión selectiva de células GABAérgicas en SM afecta el aprendizaje espacial en el LAM (Burjanadze et al, 2015), aunque en otro estudio no se ha mostrado efecto sobre el aprendizaje (Pang et al, 2001). Con relación a lo anterior, el septo medial a través de las células GABAérgicas, colinérgicas y glutamatérgicas es importante para la generación de la actividad theta (Young y Jackson, 2011).

Se ha propuesto un modelo de interacción septo-hipocampal para la generación de la actividad theta de CA1 del cuerno de Ammón. Este se refiere a que durante la actividad theta las células piramidales oscilan entre el estado de despolarización (modo de disparo) e hiperpolarización (modo inter-disparo) y la alternancia de ambos estados da lugar a flujos de corriente extracelular responsables de la generación de la actividad theta (Vertes y Kocsis, 1997).

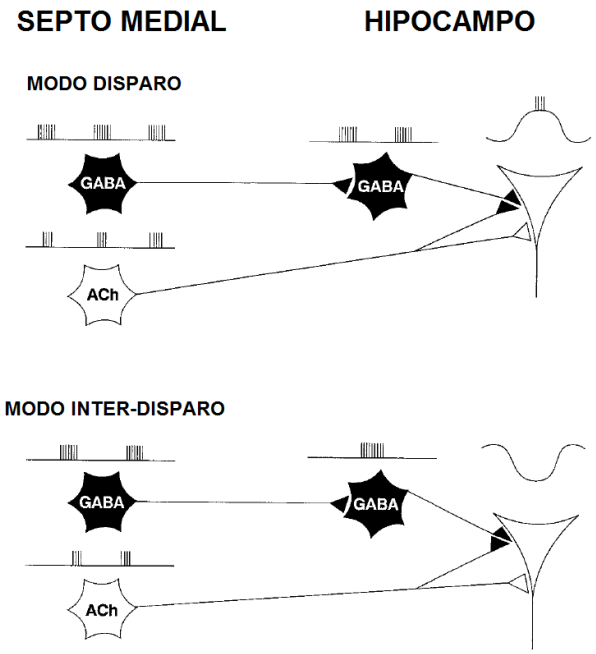


Figura 5. Modelo de la interacción septo-hipocampal para la generación de la actividad theta hipocampal. Negro es inhibitorio; blanco es excitador. Neuronas GABAérgicas (GABA) y colinérgicas (ACh) del SM disparan rítmicamente en ráfaga con theta. La descarga de neuronas GABA y ACh septales son sincrónicas, su efecto post sináptico en el hipocampo es sincrónico. Las células GABAérgicas septales terminan sobre GABA hipocampales y las células ACh septales inhiben pre sinápticamente interneuronas piramidales del hipocampo. Modificado de Vertes y Kocsis, 1997.

En la modalidad de disparo, neuronas colinérgicas del SM activan las células piramidales (CPs) e inhiben presinápticamente interneuronas hipocampales, mientras que las células GABAérgicas del SM solamente inhiben interneuronas del hipocampo. La acción coordinada de células septales colinérgicas/GABAérgicas sobre las CPs (excitación colinérgica acoplada con una desinhibición GABAérgica) excita fuertemente a las CPs. Esto lleva a la despolarización, la activación de corrientes intrínsecas de sodio y la activación de potenciales de membrana despolarizantes y espigas en las CPs que correspondería a la formación del pico de fase positiva de la onda theta (Vertes y Kocsis, 1997). En la modalidad inter-disparo (el cese del disparo de las células colinérgicas/GABAérgicas septales entre ráfagas) las células colinérgicas del SM ya no activan a las CPs o inhiben a las interneuronas hipocampales, y las células GABAérgicas septales no inhiben a las interneuronas del hipocampo dando por resultado una disminución de la influencia excitadora y un incremento en la influencia inhibitoria sobre las CPs, debido a la supresión de la acción GABAérgica septal sobre interneuronas del hipocampo. Como resultado las interneuronas hipocampales disparan, inhiben rítmicamente a las CPs, así como hiperpolarizan y bloquean la descarga inhibiendo a las CPs. Esto correspondería a la fase negativa de la onda theta (Vertes y Kocsis, 1997) (Figura 5).

A diferencia del modelo anterior, recientemente Huh (2010) propuso un modelo en el que incluyó la participación de las neuronas glutamatérgicas septales. En el modelo las neuronas colinérgicas del SM pueden proporcionar excitación a largo plazo a las células piramidales e interneuronas, mientras que las neuronas GABAérgicas del SM transmiten la inhibición rítmica únicamente a interneuronas GABAérgicas (Toth et al, 1997). Los autores proponen que las neuronas glutamatérgicas del SM inducen una despolarización rápida que puede desencadenar el disparo de espigas en las células piramidales del hipocampo. Si estas despolarizaciones son rítmicas, podrían proporcionar una entrada de sincronización potente y contribuir potencialmente a la oscilación theta hipocampal (Huh et al, 2010). Se ha mostrado que neuronas glutamatérgicas del SM pueden excitar rítmicamente neuronas vecinas dentro del SM (Manseau et al, 2005). Así, el

papel de las neuronas glutamatérgicas puede contribuir a conducir una excitación rítmica en la red local, así como en el hipocampo (Huh, et al 2010). Figura 6

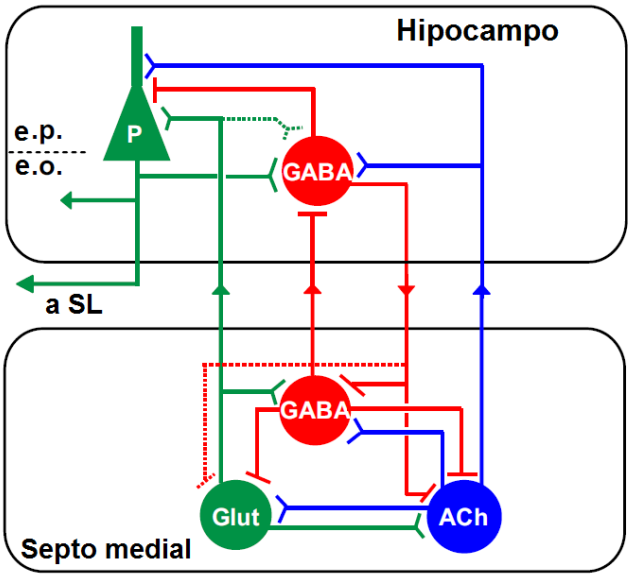


Figura 6. Un nuevo modelo de la red septo-hipocampal. Neuronas glutamatérgicas pueden servir como un generador de ritmo intrínseco que puede contribuir a la conducción excitadora rítmica de la red septal local y del hipocampo. SL, septo lateral. e.p. estrato piramidal. e.o. estrato oriens. Tomado de Huh et al, 2010.

Con lo anterior, algunos autores sugieren que la interacción entre las propiedades intrínsecas de la membrana de las diferentes poblaciones neuronales del SM y la conectividad entre ellas mismas subyace a la capacidad de la red del SM para contribuir de forma crítica al ritmo theta del hipocampo (Leão RN et al, 2015). Por lo tanto, el SM es uno de varios generadores rítmicos extrínsecos que amplifican y regulan el generador de actividad theta intrínseco dentro del hipocampo. Así, la actividad theta hipocampal registrada *in vivo* puede ser producto de varios generadores theta intrínsecos y extrínsecos interactuando y trabajando en concierto (Huh et al, 2010).

2.3 SISTEMA SEPTO-MAMILAR

El SM tiene también conexiones descendentes hacia el área mamilar, principalmente al núcleo supramamilar (SUM) y al núcleo mamilar medial de los cuerpos mamilares (MM). Las neuronas GABAérgicas del SM envían terminales

hacia neuronas aspartato/glutamatérgicas del SUM (Leranth et al, 1999). Otras células del SUM también reciben fibras de neuronas colinérgicas del SM (Gonzalo Ruiz et al, 1999). A su vez, las neuronas glutamatérgicas del SUM inervan a neuronas colinérgicas y GABAérgicas del SM, y a células granulares y principales del hipocampo (Leranth et al, 1999; Pan y McNaughton, 2004).

El SM envía información hacia el núcleo mamilar medial (MM) (Gonzalo-Ruiz et al, 1992), y puede influir directamente sobre la función de los cuerpos mamilares en relación con la actividad theta y la memoria episódica (Aggleton y Brown, 1999). Neuronas glutamatérgicas del SM hacen sinapsis con neuronas glutamatérgicas del núcleo mamilar medial (Gonzalo Ruiz et al, 1999).

Las conexiones reciprocas ya mencionadas del SM con el hipocampo y del SM con el área mamilar, permiten a grupos de neuronas septales integrar información ascendente y descendente determinante para la actividad theta (Vertes y Kocsis, 1997; Hasselmo et al, 2002) por lo que un bloqueo o alteración de la transmisión neuroquímica de estos circuitos, podría interferir con la sincronización de la actividad neuronal en el SM y así influir en la función hipocampal.

Las conexiones colaterales de las neuronas del SUM que alcanzan al complejo SM y a la formación hipocampal podrían jugar un papel importante en el control directo de la actividad theta (Vertes y McKenna, 2000).

2.3.1 Núcleo Supramamilar

El núcleo supramamilar (SUM) tiene influencia tanto directa como indirecta sobre la actividad theta del hipocampo. La influencia directa ocurre a través de sus proyecciones excitadoras (glutamatérgicas) sobre las células granulares del giro dentado, así como a las células piramidales y GABAérgicas del hipocampo (Kiss et al., 2000), indirectamente hace sinapsis sobre las neuronas colinérgicas y GABAérgicas del SM (Leranth y Kiss, 1996) que a su vez proyectan al hipocampo (Kiss et al, 2000). Se ha demostrado que la estimulación eléctrica o bien la inyección de carbacol en el núcleo reticular pontis oralis (RPO) de ratas anestesiadas, activa neuronas del SUM que disparan ráfagas sincrónicas en fase con la actividad theta hipocamapal (Kirk y McNaughton, 1991; Kirk y McNaughton, 1993; Kocsis y Vertes,

1994; Bland et al, 1995), y que la estimulación del SUM produce actividad theta hipocampal (Vertes, 1981; Oddie et al, 1994), por lo que se ha propuesto que éste núcleo actúa como un relevo en el procesamiento de información ascendente hacia el hipocampo.

Diferentes estudios han evaluado la contribución específica del SUM en la generación y control de la actividad theta, entre ellos el trabajo de Kirk y McNaughton (1993) en el que realizaron inyecciones de procaína en varias regiones del SUM en ratas anestesiadas y observaron que se redujo la frecuencia y la amplitud de la actividad theta generada mediante la estimulación del RPO (Kirk y McNaughton, 1993). Estos autores observaron además que cuando ocurre un bloqueo temporal especifico del SM la actividad de las células del SUM no se altera, pero la amplitud de la actividad theta en el hipocampo se reduce, mientras que cuando la inyección se hace dentro de sitios entre el RPO y el SUM se reduce la frecuencia de la actividad theta, pero no la amplitud (Kirk y McNaughton, 1993). Por otro lado, Lee et al (1994), mostraron que la inyección de la neurotoxina colinérgica 192 inmunoglobulina G-saporina en el SM reduce significativamente la amplitud, pero no la frecuencia de la actividad theta (Lee et al, 1994). Con base a estos antecedentes se ha propuesto que la frecuencia de la actividad theta es codificada en el SUM, es decir que la transducción de la intensidad de la activación reticular a la frecuencia de la actividad theta resultante, tiene lugar en tal región, y que la señal que codifica la frecuencia (entrada fásica) es enviada probablemente a través del haz medial frontocerebral (MFC) hacia el MS (Kirk, 1998; Kirk y McNaughton, 1993), aunque actualmente no se conoce la vía anatómica. En apoyo a la existencia de dicha influencia, hay evidencias experimentales de que la estimulación del SUM lateral mediante la aplicación de glutamato en ratas anestesiadas genera un incremento en la población de espigas en el GD evocadas por estimulación de la vía perforante, por lo cual se ha propuesto que las aferentes del SUM lateral constituyen un mecanismo para la modulación heterosináptica de corta y larga duración en el hipocampo (Nakanishi et al, 2001). De acuerdo con ello, la co-activación del SUM y de la vía perforante produce potenciación de larga duración (PLD) de la población de espigas de las células granulares y dicho PLD es abolido

cuando se lesionan las vías aferentes del SUM al hipocampo (Nakanishi et al, 2001), de esta manera es evidente que el SUM podría estar contribuyendo al procesamiento de la memoria asociativa a través de la modulación de la excitabilidad hipocampal, sin embargo, esto aún no está comprobado.

En base a lo anterior se ha sugerido que la actividad rítmica en el SUM es requerida para la modulación de la frecuencia de la actividad theta, recibiendo altos niveles de activación del RPO que pueden ocurrir normalmente en estados de una conducta particular (por ejemplo, el aprendizaje y emoción) (Vertes, 1982) o durante estimulación del RPO, pero no sería necesaria para la expresión de theta *per se*.

2.3.2 Cuerpos mamilares (Núcleo mamilar medial)

Los cuerpos mamilares, se localizan en el margen posterior del hipotálamo, sobre la base del cerebro (Paxinos y Watson, 1998). Se dividen en el núcleo mamilar medial (MM) y núcleo mamilar lateral (ML). El núcleo medial es grande y está compuesto de entre 1-5 núcleos, tiene proyecciones ipsilaterales al núcleo talámico anterior medial y anterior ventral. El núcleo mamilar lateral, es una estructura más pequeña (Allen y Hopkins, 1988), y proyecta bilateralmente al núcleo talámico dorsal anterior. Ambos núcleos, son inervados por el núcleo supramamilar y la región septal (Gonzalo-Ruiz et al, 1992). El núcleo mamilar medial, recibe densas proyecciones del hipocampo por medio del fornix comisural (Gonzalo-Ruiz et al, 1992).

Los cuerpos mamilares constituyen un elemento clave del circuito de Papez (Papez, 1937; MacLean, 1952) y son una parte integral del circuito en el cual las señales le son transmitidas del septum (Swanson y Cowan, 1977; Gonzalo-Ruiz et al, 1992), subiculum (Swanson y Cowan, 1977) y corteza entorrinal (Shibata, 1988).

El núcleo mamilar medial (Figura 7) presenta neuronas que disparan rítmicamente en fase con la actividad theta hipocampal (Bland et al, 1995). Estas células relacionadas a theta son dirigidas por proyecciones descendentes del hipocampo y del SM; y están correlacionadas con el generador de actividad theta de CA1 (Kocsis y Vertes, 1994). Como ya se mencionó anteriormente, el SUM contribuye al control

de la frecuencia y ritmicidad theta hipocampal. En relación con esto, la inactivación septal elimina la actividad theta en los cuerpos mamilares, pero no en el núcleo supramamilar (Kirk et al., 1996), lo cual indica que los cuerpos mamilares (principalmente MM) son relevo de información descendente de la actividad theta, y se ha propuesto que podrían reducir interferencias para separar la codificación y el recuerdo (Hasselmo et al, 2002). En la Figura 7, se muestra el circuito que conecta el septo medial e hipocampo (CA1) con el núcleo mamilar medial y su participación en la actividad rítmica theta. En este circuito, la información fásica entra al septo medial que contiene neuronas GABAérgicas (G) y colinérgicas (C), a través de las conexiones inhibidoras y excitadoras del SM hacia el hipocampo, el hipocampo conduce la actividad fásica hacia los cuerpos mamilares (principalmente al MM). La amplitud de registro (septo medial, CA1 y cuerpos mamilares) varia relativamente uno con otra, lo cual indica que en el circuito ocurre un control diferente del patrón de información fásica en de cada estructura.

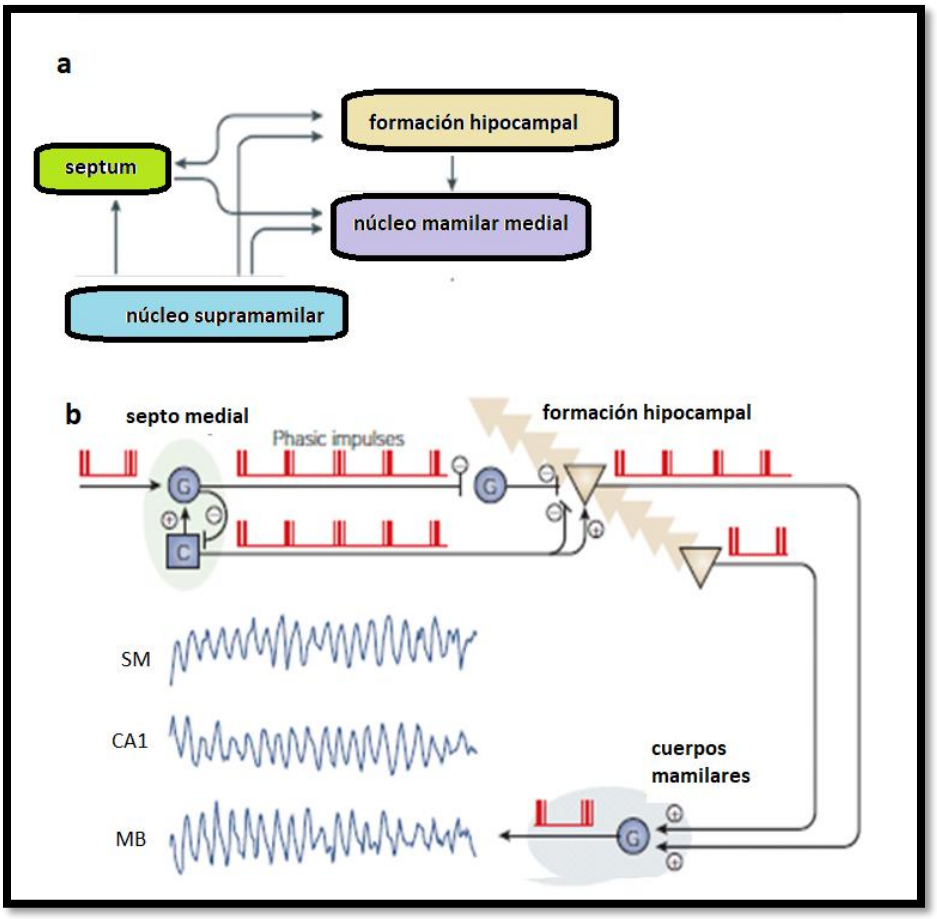


Figura 7. Núcleo mamilar medial y la actividad theta. Modificado de Vann y Aggleton, 2004.

Los cuerpos mamilares han sido relacionados directamente con procesos de memoria. Se ha observado a roedores con lesión de los cuerpos mamilares o en el tracto mamilotalámico que muestran deterioro en la adquisición de información en una tarea de alternancia espacial (Aggleton, Hunt y Shaw, 1990; Vann y Aggleton, 2003), en la memoria de trabajo espacial (Vann y Aggleton, 2003; Santín et al, 1999) y la memoria de referencia en el laberinto acuático de Morris (Sziklas y Petrides, 1998; Santín et al, 1999). De igual manera, monos con lesión de los cuerpos mamilares también son deficientes en pruebas de memoria espacial (Sziklas y Petrides, 1998). El patrón de deficiencias espaciales que está asociado con daño de los cuerpos mamilares en ratas puede ser caracterizado como una falla de la codificación rápida alocéntrica (Vann y Aggleton, 2003), que es un deterioro en la habilidad para aprender una localización especifica mediante un mapa cognitivo.

Finalmente, se ha propuesto que el área mamilar que incluye a los cuerpos mamilares y al núcleo supramamilar podrían ocupar una posición estratégica en el tráfico de información ascendente y descendente entre el tallo del cerebro y la parte límbica (septum-hipocampo) posiblemente involucrados en la activación o en el aumento de la actividad rítmica theta (Dillingham et al, 2015).

2.4 SISTEMA SEROTONINÉRGICO COMO MODULADOR DE LA ACTIVIDAD THETA HIPOCAMPAL

Existen evidencias anatómicas de aferentes serotoninérgicas hacia el hipocampo, SM (Jakab y Leranth, 1995) y hacia el área mamilar (Pan y McNaughton, 2004). Se ha propuesto que la serotonina es capaz de modular la actividad de neuronas colinérgicas y GABAérgicas del SM a través de diferentes receptores distribuidos sobre las mismas, ejerciendo un efecto inhibidor general sobre las terminales colinérgicas y GABAérgicas del hipocampo y del SM de la rata (Miettinen y Freund, 1992).

2.4.1 La Serotonina, aprendizaje y memoria espacial.

Existen evidencias que muestran poca repercusión del abatimiento de la serotonina sobre el aprendizaje y la memoria dependientes del hipocampo. Se ha observado en algunos estudios que cuando se abate o se interfiere con el sistema serotoninérgico no se afecta el desempeño del aprendizaje y la memoria espacial. Por ejemplo, luego del abatimiento de serotonina mediante la aplicación intracerebral o intra-rafé de 5,7 Dihidroxitriptamina, o cuando las ratas son tratadas con PCA (p-chloroanfetamina) (Altman et al, 1989), PCPA (chlorofenylalanina) (Richter-Levin y Segal, 1989) en pruebas del laberinto acuático de Morris y del laberinto radial de Olton se ha observado que no hay alteraciones en el desempeño cognoscitivo de la rata (Altman, 1990; Asin, Wirtshafter y Fibiger, 1985). La administración intra-hipocampal del neurotóxico con eliminación parcial tampoco genera alteraciones en la prueba del laberinto acuático de Morris (Altman et al, 1990; Olvera–Cortés, 2003). Sin embargo, la desaferentación serotoninérgica

específica del hipocampo facilita el aprendizaje de discriminación espacial con reforzamiento positivo (Alman et al., 1990) y la eliminación severa (98-100%) de la serotonina involucrada en procesos neurales específicos del hipocampo ocasiona una facilitación en el aprendizaje espacial dependiente del hipocampo (Gutiérrez-Guzmán et al, 2011). Altman (1990), sugiere que la serotonina ejerce una influencia negativa sobre una o más vías hipocampales críticas para el procesamiento de información espacial dependiente del hipocampo.

2.4.2 La serotonina como desincronizador de la actividad theta

Se ha propuesto que el sistema serotoninérgico originado en los núcleos del rafé está involucrado directamente con la desincronización o estado no-theta de la actividad eléctrica hipocampal (Vinogradova, 1995; Vertes and Kocsis, 1997; Leranth and Vertes, 2000; Vertes, 2010). Esto se ha derivado de experimentos en los cuales se ha observado que cuando se estimula al rafé mesencefálico ocurre una inhibición en la actividad theta hipocampal (Vertes et al, 1986), además, la estimulación eléctrica del rafé medial (RM) inhibe el disparo de células piramidales hipocampales (Segal, 1975) y desincronizan la actividad eléctrica hipocampal (Kitchigina et al, 1999; Vertes, 1981). En otros estudios la lesión específica del RM produce la presencia de actividad theta hipocampal continua y persistente (Yamamoto et al, 1979; Maru, Takahashi y Shinkuro, 1979*;* Vertes, 1986; Vinogradova, 1995). En contraste, se ha mostrado que la inhibición de neuronas serotoninérgicas por agonistas selectivos al receptor 5HT1A induce actividad theta hipocampal en gatos en libre movimiento (Marrosu, Fornal, Metzler y Jacobs, 1996), y la aplicación de agonistas al receptor 5HT1A en el RM que inhibe el disparo de las neuronas del rafé, genera trenes largos y continuos de actividad theta en ratas anestesiadas (Vertes et al, 1994), mientras que la disminución de serotonina hipocampal incrementa el tamaño de la población de espigas en GD (Richter-Levin, Greenberger y Segal, 1994). La administración sistémica del agonista al receptor 5HT2C inhibe las oscilaciones theta del SM y la actividad theta en el hipocampo, mientras que cuando se aplican antagonistas selectivos al receptor 5HT2C, dicha inhibición se revierte y se induce actividad theta hipocampal, en ratas anestesiadas

(Hajos, Hoffmann y Weaver, 2003), por lo tanto, este receptor participa en la mediación de la acción inhibitoria de la serotonina sobre la actividad theta. También se ha reportado que la activación del receptor 5HT1A en el SM deteriora el desempeño en el laberinto acuático de Morris (Koenig et al, 2008). Estos receptores son expresados en 98% de las neuronas GABAérgicas positivas a parvalbumina del MS (Luttgen et al, 2005), por lo cual la serotonina puede estar modulando la actividad de las neuronas GABAérgicas.

En relación con lo anterior, Vinogradova y colegas concluyen que: “el núcleo de rafé medial puede ser considerado como un antagonista funcional de la formación reticular, suprimiendo de manera muy eficiente el disparo theta de neuronas del área septal medial y la actividad theta hipocampal” (Vinogradova et al, 1999).

Como ya se ha mencionado, la actividad theta se presenta durante conductas asociadas a la adquisición de información, y tiene un papel muy importante en la potenciación de larga duración. A partir de lo anterior, algunos autores sugieren que la serotonina media la desincronización de la actividad theta suprimiendo la PLD y la memoria (O’Keefe y Nadel, 1978; Staubli y Otaky, 1994), es decir, la serotonina podría bloquear o suspender temporalmente los procesos mnémicos del hipocampo (Vertes, 2005). En relación con esto, se ha propuesto que la información que llega al hipocampo, durante estados en que predomina la actividad theta, es codificada y almacenada por lo menos temporalmente en el mismo, mientras que aquella información que llega en ausencia de actividad theta no es codificada; al menos no al mismo grado que alcanza la información que llega al hipocampo concurrente con la actividad theta (Vertes y Kocsis, 1997). Con ello se ha sugerido que el sistema serotoninérgico originado en el rafé medial puede ser una parte importante del sistema directo de conexiones al hipocampo, hasta cierto punto encargado de la capacidad de ignorar eventos ambientales no significativos (Vertes, 2005), como un mecanismo de selectividad de información para codificar solo la información relevante. En apoyo a esto, se ha propuesto que cuando el tono serotoninérgico en el SM es alto, disminuiría la liberación de acetilcolina y GABA al hipocampo, lo cual, evitaría la codificación de información; y un bajo tono serotoninérgico en el SM

incrementaría la liberación de acetilcolina y GABA, lo cual permitiría una buena codificación y consolidación de información (Jeltsch-David et al, 2008). Figura 8.

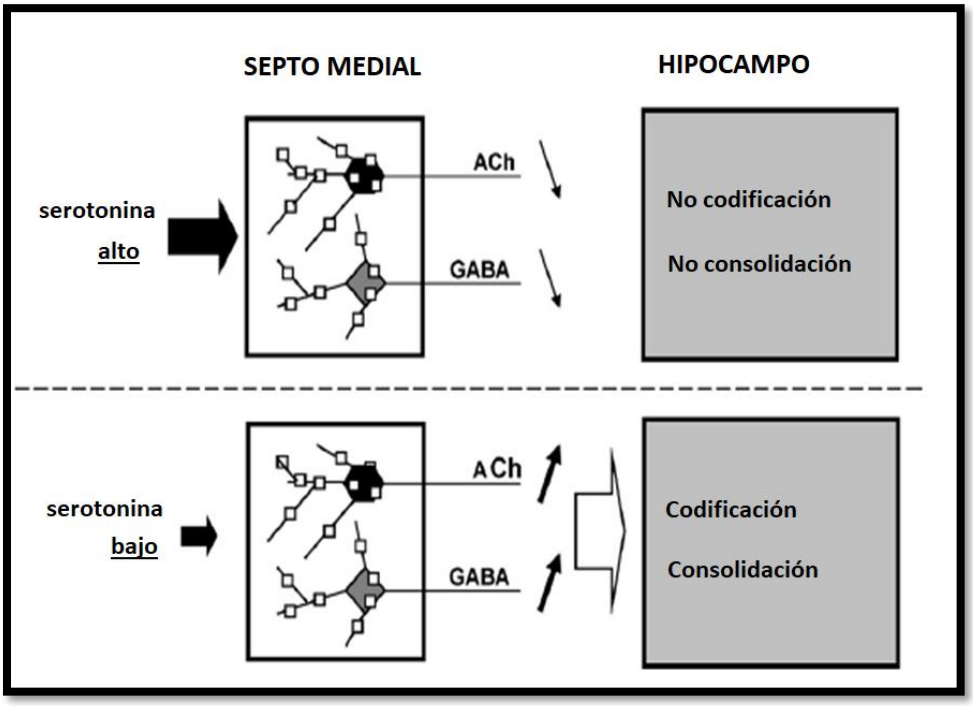


Figura 8. Representación esquemática acerca de la participación serotoninérgica a nivel septal para modular la codificación y consolidación de información. Modificado de Jeltsch-David et al, 2008.

Sin embargo, un punto interesante es que la mayoría de los estudios con respecto a la serotonina como desincronizador de la actividad theta hipocampal, han sido realizados principalmente en animales anestesiados y a partir de ellos, se han hecho inferencias o planteado hipótesis acerca de la implicación funcional que podría tener un estado de alto o bajo tono serotoninérgico hacia el hipocampo y áreas relacionadas como el septo medial facilitando o no la codificación de información; y no han sido confirmados experimentalmente durante conductas que demanden el procesamiento de información a través de diferentes días.

En relación con lo anterior, son pocos los trabajos en los que se muestra el posible papel de la serotonina en la modulación de la actividad theta hipocampal en relación con el procesamiento de información (Olvera-Cortés et al, 2013), sin embargo, esto no ha sido completamente aclarado. Se ha observado que la eliminación de la

serotonina del hipocampo produce una facilitación en el aprendizaje asociado con un aumento de la potencia theta de alta frecuencia (6.5-9.5 Hz) (Gutiérrez-Guzmán et al, 2011). Sin embargo, se ha observado que una reducción de la serotonina del SUM y del núcleo hipotalámico posterior ocasiona una menor eficiencia para resolver una tarea de aprendizaje y memoria espacial en el laberinto acuático de Morris, asociado a la ausencia de cambios relacionados al aprendizaje en la actividad theta hipocampal (Gutiérrez-Guzmán et al, 2012). La reducción de serotonina especifica del SUM produce deficiencias en el aprendizaje asociadas con una comunicación reducida entre el SM y el área CA1 del hipocampo (Hernández-Pérez et al, 2015).

Así, dada la importancia del septo medial sobre la función hipocampal y la serotonina como modulador del aprendizaje y actividad theta hipocampal, sería importante conocer el papel funcional de la serotonina sobre el septo medial y su repercusión sobre la actividad theta que ocurre durante conductas que implican la codificación y recuperación de la información, como en el aprendizaje y la memoria que depende de la función del hipocampo. También es importante conocer que implicación funcional podría tener la serotonina en el SM sobre los núcleos del área mamilar, ya que estos tienen conexión reciproca con el septo medial y particularmente el núcleo supramamilar codifica la frecuencia de la actividad theta y envía información fásica hacia el septo medial, de manera que el SM integra información que recibe de este núcleo. A su vez, el núcleo mamilar medial integra información descendente del hipocampo y la envía al núcleo supramamilar siendo importante para la codificación de información espacial y actividad theta relacionada con CA1 del hipocampo.

Estudiar estos circuitos complejos, septo-hipocampo y septo-mamilar, nos ayudara a conocer la relación funcional de estas áreas durante el procesamiento de información espacial, y cómo la serotonina podría estar modificando la función, a través del septo medial, ya que estarían implícitos diferentes estados del procesamiento de información en cada nivel de los circuitos.

3. PLANTEAMIENTO DEL PROBLEMA

El SM tiene un papel importante en la coordinación temporal del flujo de información hacia el hipocampo y la generación de la actividad theta hipocampal, así como en el aprendizaje espacial. El SM es una estación crítica en el circuito que conecta el hipocampo y otras regiones subcorticales como los núcleos SUM que participa en la codificación de la frecuencia de la actividad theta hipocampal y el MM que integran y conducen información descendente del hipocampo. El SM y el hipocampo reciben densa inervación serotoninérgica originada en los núcleos de rafé y expresan diferentes tipos de receptores a la serotonina. Se ha propuesto que el sistema serotoninérgico actúa como un desincronizador (o estado no-theta) del EEG hipocampal ya que una supresión de la actividad serotoninérgica produce una actividad theta continua y persistente en contraste con la pérdida de la actividad rítmica theta del hipocampo por aumento de la actividad serotoninérgica. Además, se ha sugerido que un bajo tono de serotonina hacia el SM facilitaría la codificación de información, mientras que alto tono serotoninérgico hacia SM impediría la codificación y consolidación de información.

Un punto interesante es que la mayoría de los estudios con respecto a la serotonina como desincronizador de la actividad theta hipocampal, han sido realizados principalmente en animales anestesiados y a partir de ahí, se han hecho inferencias acerca de la implicación funcional que podría tener un estado de alto o bajo tono serotoninérgico hacia el hipocampo y áreas relacionadas como el septo medial.

Debido al importante papel del SM sobre la actividad theta y el aprendizaje espacial, así como a la participación de la serotonina como posible desincronizador de la actividad theta hipocampal, es importante conocer el papel funcional de la serotonina en el septo medial y su repercusión sobre la actividad theta del circuito septo-hipocampo y septo-mamilar, específicamente durante conductas que involucran la codificación y recuperación de información, como el aprendizaje y la memoria.

La reducción o aumento de la actividad serotoninérgica hacia el SM podría interferir con la actividad septal y a su vez influir en la actividad theta del hipocampo y área

mamilar, dado el papel regulador del septo y núcleos mamilares sobre la fisiología hipocampal. Por lo anterior, se pretende conocer, cuál es la relación de la actividad theta del septo medial con la del hipocampo y región mamilar durante una prueba de aprendizaje espacial, en condiciones de modificación de la actividad de la serotonina septal, en la rata.

4. OBJETIVOS

- Determinar el efecto de la reducción de la serotonina del septo medial, sobre la actividad theta del septo medial y su repercusión sobre la actividad theta del hipocampo y de los núcleos supramamilar y mamilar medial, durante una prueba de aprendizaje y memoria espacial que depende del hipocampo.

- Determinar el efecto de la reducción de la serotonina del septo medial, sobre el aprendizaje y la memoria espacial evaluada en el laberinto acuático de Morris.

- Determinar el efecto del aumento de la serotonina septal, sobre el aprendizaje y la memoria espacial evaluada en el laberinto acuático de Morris.

5. HIPÓTESIS

- La reducción de la serotonina septal ocasionará cambios en la actividad theta hipocampal.

- La reducción de serotonina septal ocasionará cambios en la actividad theta de los núcleos mamilares.

- Los cambios de la actividad theta hipocampal estarán asociados con el desempeño en el aprendizaje espacial.

- La reducción de la serotonina del septo medial producirá una facilitación en el aprendizaje espacial evaluado en el laberinto acuático de Morris.

- El aumento de la serotonina del septo medial ocasionará deficiencias en el aprendizaje espacial evaluado en el laberinto acuático de Morris.

6. METODOLOGÍA

Se utilizaron 49 ratas macho de la cepa Sprague-Dawley de 400 a 460 g de peso, mantenidas en un bioterio bajo condiciones estándar con un ciclo normal de luz oscuridad de 12 por 12 horas (7-19 hrs), con temperatura de 22 °C y libre acceso al alimento y agua. Las ratas fueron asignadas al azar a los diferentes grupos.
Los procedimientos experimentales se realizaron de acuerdo con la Norma Oficial Mexicana (NOM-062-ZOO-1999) de especificaciones técnicas para la producción, cuidado y uso de los animales del laboratorio y con la guía de los Institutos de Salud de los Estados Unidos de Norteamérica (NIH) para el Cuidado y Uso de Animales de Laboratorio (Publicación NIH No.80-23, 1996). Todos los experimentos fueron aprobados por el Comité de Ética en Investigación del Instituto Mexicano del Seguro Social.

6.1 Procedimiento de cirugía estereotáxica

Las ratas fueron anestesiadas mediante la aplicación de Ketamina (60 mg/kg) vía intramuscular (im) y la administración de Pentobarbitál Sódico (14 mg/kg) por vía intraperitoneal (ip). **Las ratas del grupo 5HT-D (n=8),** se sometieron a la reducción de la serotonina del septo medial a través de la micro-infusión del neurotóxico 5,7-dihidroxitriptamina (5,7-DHT) que destruye terminales sinápticas serotoninérgicas (1. 5 µg de 5,7-DHT disuelto en 0.1µl de Ácido Ascórbico en solución salina) a una velocidad de flujo 0.1 µl/min durante 5 minutos mediante el uso de una Jeringa Hamilton 10-µl y una bomba de infusión. Media hora antes de realizar la aplicación del neurotóxico, los animales recibieron 30 mg/kg de Pargilina (ip), para proteger las terminales noradrenérgicas y evitar la captura del neurotóxico (Breese y Cooper, 1975; Breese et al., 1978; Oreland et al., 1973; Chuang et al., 1974). **Las ratas del grupo Vehículo (n=8),** recibieron una micro-infusión de solución vehículo en volumen y velocidad similar al grupo 5HT-D. Las coordenadas estereotáxicas para la micro-infusión sobre septo medial fueron basadas en el atlas de Paxinos and Watson (1998): 0.6 mm anterior a Bregma (AB), 1.5 mm lateral a la línea media (LM)

y 6.6 mm dorsoventral a la superficie del cráneo (DVC) con un ángulo de 15º desde la vertical. De las 49 ratas, 29 fueron asignadas para los grupos 5HT-D y vehículo, de las cuales se descartaron 13 ratas por no cumplir los criterios de inclusión que se describen en la sección de resultados.

El grupo CITAL (n=11), fue formado con ratas a las que se les aumentó la serotonina del septo medial mediante la infusión de citalopram (un inhibidor selectivo de la recaptura de serotonina) dentro del SM. Durante la cirugía se realizó el implante crónico de una cánula guía calibre 23 que sirvió para orientar el trayecto de otra cánula interna a través de la cual se infundió el citalopram antes del entrenamiento conductual durante cada día de prueba. Media hora antes de la prueba conductual, las ratas recibieron la infusión de 0.6-0.8 µl de citalopram (0.1 M en líquido cefalorraquídeo artificial, velocidad de flujo 0.2 µl/min) a través de una cánula de calibre 30, insertada dentro de la cánula guía calibre 23 cuyo extremo estaba colocado 2 mm debajo del cráneo orientada para facilitar la localización del septo medial y ubicar el extremo de la cánula interna, de acuerdo a las coordenadas estereotáxicas: 0.5 mm AB, 0 mm LM y 6.6 DVC. La cánula interna fue conectada a una bomba de infusión con una jeringa Hamilton de 10µl. **Al grupo LCFA (n= 9)** se le aplicó de la misma manera solución vehículo (líquido cefalorraquídeo artificial) con volumen y velocidad similar al de aplicación de la solución de citalopram. Luego de 10 días de recuperación de la cirugía, las ratas fueron evaluadas en la tarea de aprendizaje espacial en el laberinto acuático de Morris (no se realizó registro de la actividad theta). Dos días antes del inicio de estas pruebas, las ratas fueron manipuladas (llevadas al cuarto donde se aplicaría el fármaco, simulación de colocación y retiro de aguja de infusión) para reducir el estrés que pudiera generarse durante el procedimiento de aplicación del fármaco.

En el mismo procedimiento quirúrgico, solo a las ratas de los grupos 5HT-D y Vehículo se les realizó el implante crónico de los 5 electrodos de registro (Figura 9). De acuerdo con el atlas de Paxinos y Watson (1998), los electrodos del hipocampo se localizaron en el estrato oriens/piramidal del área CA1 del hipocampo dorsal derecho con coordenadas esterotáxicas 4.5 mm posterior a Bregma (PB), 2.5 mm

LM y 2.4 mm DVC y en la región del giro dentado con coordenadas 3.5 mm PB, 1.5 mm LM y 3.4 mm DVC. Un electrodo estuvo localizado en el septo medial con las mismas coordenadas a la aplicación del 5,7-DHT. Dos electrodos se localizaron en la región mamilar (núcleo supramamilar, coordenadas 4.6 mm AP, 0 mm LM y 8.2 mm DVC; núcleo mamilar medial, coordenadas 4.6 mm AP, 1.3 mm LM y 9.2 mm DVC con un ángulo de 13º desde la vertical). Los electrodos bipolares fueron construidos con alambre de nicromo de 60 µm de diámetro, fijado dentro de una cánula de acero inoxidable calibre 30 y aislada con resina epóxica. Se dejó expuesta una punta del electrodo con una pequeña superficie de registro. Se colocó un tornillo de acero inoxidable sobre el hueso del cráneo que funciono como tierra. Una vez terminada la cirugía tanto de lesión como del implante de electrodos, se aplicó un antibiótico (30mg/kg) y analgésico intramuscular. Posterior a 15 días de recuperación quirúrgica, se evaluó a los animales en una prueba del laberinto acuático de Morris con un registro simultáneo de la actividad eléctrica durante 7 días de entrenamiento.

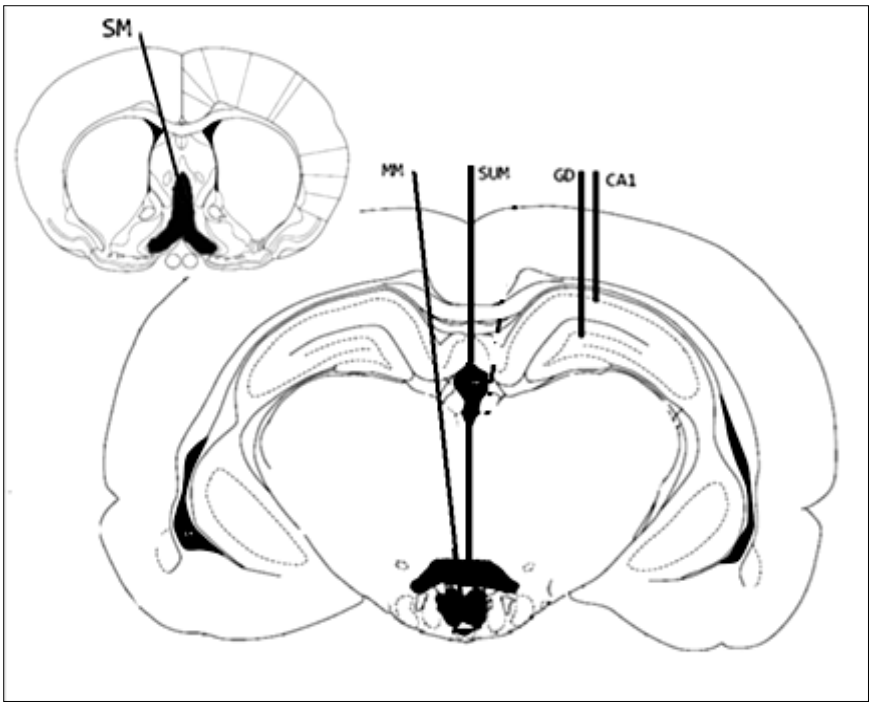


Figura 9. Esquema representativo (corte coronal) del sitio de aplicación de los fármacos en el SM, la colocación de los electrodos en el hipocampo dorsal (GD, CA1) y área mamilar (SUM, MM). Modificado de Paxinos y Watson, 1998.

6.2 Evaluación conductual

Para evaluar el aprendizaje espacial se utilizó el laberinto acuático de Morris (Figura 10) que consiste en una tina circular de 150 cm de diámetro llena con agua mantenida a 27 +-1 °C y teñida en azul obscuro para evitar que la rata vea una plataforma circular de 10 cm de diámetro que se mantuvo 2 cm bajo la superficie del agua y colocada en una posición fija en un solo cuadrante, además se colocaron señales espaciales en el cuarto de registro, alrededor del laberinto. Las ratas tenían que aprender a localizar la plataforma de escape.

La prueba consistió en 4 ensayos diarios con intervalo inter-ensayo de 2 minutos durante 6 días consecutivos. Si durante el transcurso de cada ensayo la rata no encontraba la plataforma en 60 segundos, se le colocaba sobre la plataforma donde permanecería por un periodo de 15 segundos. Con el objetivo de evaluar el grado de aprendizaje y determinar el tipo de estrategia utilizada para resolver la prueba, durante el día 7 se realizó un ensayo de prueba, el cual consistió en colocar al animal en la tina sin la plataforma durante un periodo de 30 segundos. Durante cada ensayo se soltó a los animales aleatoriamente de diferentes puntos y de cara a la pared de la tina.

Las rutas de nado fueron grabadas con una cámara de video y simultáneamente se trazaron las rutas mediante el programa video-bench (Data Wave Technologies) que calcula la distancia, latencia, velocidad, entre otras variables conductuales.

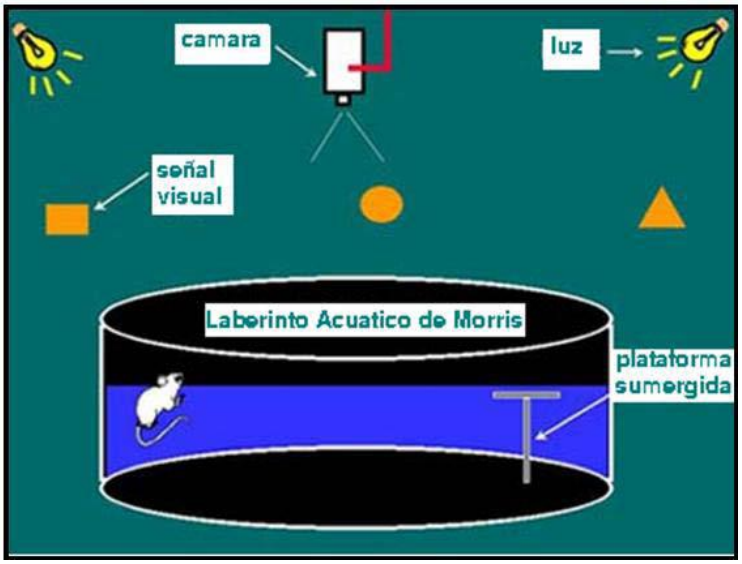


Figura 10. Representación esquemática del cuarto donde se evaluó a las ratas en el laberinto Acuático de Morris. Modificado de http://www.mcg.edu/Core/Labs/sabc/Morriswatermaze.htm

Para el análisis se consideró la distancia total recorrida, la latencia de escape (tiempo que toma el animal en localizar la plataforma) y la velocidad de nado de cada animal durante la búsqueda de la plataforma por cada ensayo y por día de entrenamiento. También, en el ensayo de prueba del día 7 (30 segundos sin plataforma) se analizó el número de cruces por el área del cuadrante y por anillo de la plataforma en el cuadrante donde se ubicaba la plataforma.

6.3 Registro de la actividad eléctrica

Durante la ejecución de la prueba conductual se realizó el registro simultáneo de la actividad eléctrica de las diferentes regiones.

Antes del inicio del primer ensayo de cada día de entrenamiento se realizó el registro en estado *basal* (atento-quieto) con la rata mojada en su caja de reposo durante 60 segundos; durante el estado de *búsqueda* se registró desde la colocación de la rata en el agua hasta que subió a la plataforma de escape en un tiempo no mayor a 60 segundos, momento a partir del cual se registraron 15 segundos sobre la plataforma, lo que correspondió al estado *meta*. Al séptimo día se registró durante el único ensayo de 30 segundos sin plataforma.

Las ratas fueron conectadas a un conmutador (Neuro-Tek, CA. IT) usando un cable con conector macho. El conmutador fue conectado a un amplificador (Sistema de adquisición Neurodata, GRASS Mod 15A54, Astro Med Inc. 600 E. Greenwich Ave., W. Warwick, RI 02893, USA). La señal registrada fue digitalizada a una frecuencia de muestreo de 1024 Hz (Sistema de adquisición de datos Data Wave Technologies) y filtro pasa-banda de 1-100 Hz. Se aplicó un filtro notch a 60 Hz para eliminar ruido de línea. La señal fue almacenada en una computadora para ser analizada fuera de línea. Los datos fueron importados a MATLAB (Mathworks, Inc). Los segmentos con artefactos (causados principalmente por sacudidas de cabeza) fueron visualmente identificados y eliminados usando el software EEGLAB (Delorme y Makeig, 2004).

Antes de iniciar el primer día de entrenamiento, se realizó el registro de la señal electrica durante la condición basal (atenta-quieta) con la rata en su caja por un periodo de 60 segundos. Posteriormente se registró durante la búsqueda de la plataforma de cada ensayo. La señal de cada región fue analizada mediante la Transformada Rápida de Fourier. La potencia absoluta fue obtenida del espectro promedio de muestras de 2 segundos para tener una resolución de 0.5 Hz. La potencia relativa (PR) se obtuvo para cada 0.5 Hz de frecuencia como el porcentaje del total de la potencia de la banda de 4-12 Hz. Se determinó el promedio de la PR a cada frecuencia de los cuatro ensayos de cada día de prueba.

La coherencia fue determinada entre el SM y el hipocampo (SM-GD, SM-CA1) y entre el SM y los núcleos mamilares (SM-SUM, SM-MM) a través de los diferentes días de entrenamiento. Los valores de la potencia y coherencia fueron obtenidos utilizando un programa personalizado adaptado de la librería MATLAB escrita por Ken Harris, disponible en: http://osiris.rutgers.edu/Buzsaki/software.

6.4 Neuroquímica- concentración de serotonina

Posterior al último día de prueba, las ratas del grupo 5HT-D y Vehículo fueron decapitadas para determinar la concentración de la serotonina y su metabolito el Ácido 5-Hidroxi-indol acético (5HIAA) del tejido septal, mediante la técnica de cromatografía liquida de alta resolución. Se realizó la extracción de una rebanada

de cerebro y con una punta de micropipeta se extrajo selectivamente el tejido del septo medial. Las muestras de tejido fueron homogenizadas en HCl 1N, se centrifugaron, se retiró el sobrenadante y fue filtrado. La concentración de la serotonina y 5HIAA fue determinada usando una columna Lichrocart purospher (150 _ 4.6, RP – 18, 5 mm, MERK KGa A, Darmstadt; Germany) y un detector electroquímico (AtecLydenVT-03) con un potencial de trabajo de 0.800 mV ajustado a un pH de la fase móvil. La fase móvil fue elaborada con ácido cítrico (50 mM), H_3PO_4 (50 mM), EDTA (20 mg), ácido octasulfonico (120 mg/L) y metanol (10 %) a pH 3.1. La velocidad de flujo fue de 1.5 mL/min. La concentración fue expresada en pg/mg.

Durante el proceso de extracción del tejido septal fue verificada visualmente la posición correcta de los electrodos del SM, mientras que resto del cerebro fue colocado en una solución fijadora por varios días para verificar histológicamente la posición de los electrodos en las otras regiones de registro (GD, CA1, SUM, MM). Se realizaron cortes histológicos de 30 micras a través de un microtomo y fueron teñidos con violeta de cresilo.

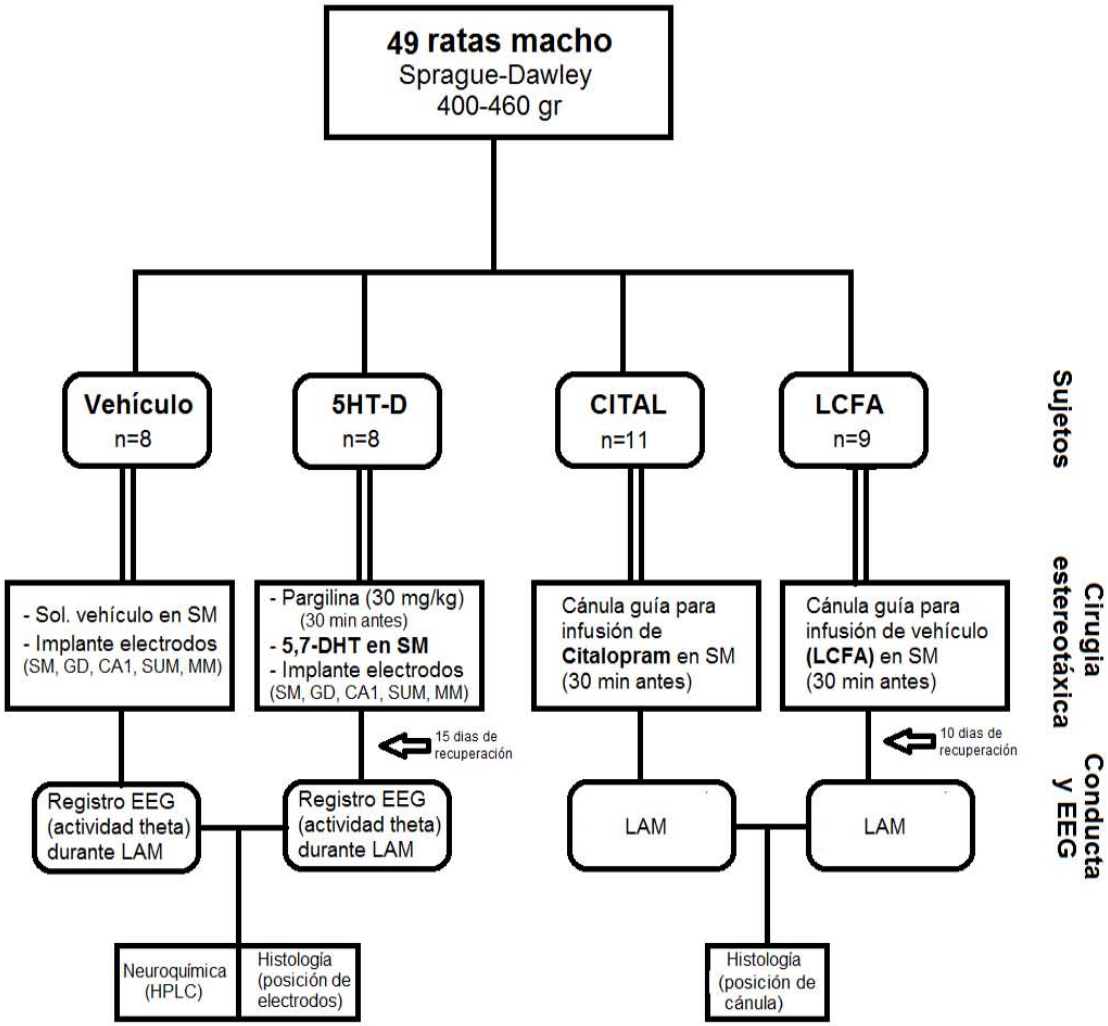
6.5 Diseño estadístico

La distancia total recorrida y la velocidad de nado fueron analizadas por día de entrenamiento mediante un ANOVA para medidas repetidas y prueba-t con ajuste Bonferroni como *pos-hoc.* Para el análisis inter-grupal se realizó un ANOVA de dos vías para medidas repetidas, con factores grupo y día, para cada etapa conductual. La comparación intra-grupal de la latencia de escape se realizó de la mediana promedio por día, aplicando el análisis de varianza de Friedman y la prueba de rangos señalados y pares igualados de Wilcoxon. Para la comparación inter-grupal se aplicó la prueba de Kruskal-Wallis y la U de Mann- Withney.

Par comparar el número de cruces por el anillo central de cada cuadrante (equivalente a la posición de la plataforma) durante el ensayo de transferencia del día 7, se realizó un ANOVA de dos vías (grupo-cuadrante) y prueba-t con ajuste Bonferroni como *pos-hoc*.

La PR de cada región registrada fue comparada por día y frecuencia. Para la comparación intra-grupal, se obtuvo la PR en el rango de 4-12 Hz de cada región por día, frecuencia y estado conductual en cada grupo, usando ANOVA de dos vías para medidas repetidas y prueba-t, con corrección de Bonferroni como prueba post hoc. La comparación inter-grupal de la PR fue realizada usando un ANOVA para medidas repetidas considerando los factores grupo y frecuencia como independientes y días (1-6) de entrenamiento como repeticiones. La comparación de la coherencia entre la señal de las diferentes regiones se realizó de manera similar a la PR.

METODOLOGÍA GENERAL



7. RESULTADOS

A. Resultados del experimento 1: grupo 5HT-D y Vehículo

7.1 Verificación de la concentración de serotonina y la posición de los electrodos

En la Figura 11 se muestra la concentración de serotonina y su metabolito 5HIAA del tejido del septo medial en los dos grupos. Nueve ratas que no cumplieron el criterio de inclusión fueron descartadas (reducción de serotonina mayor al 50 % comparado con el grupo vehículo). En el grupo 5HT-D se obtuvo una reducción significativa en la concentración de serotonina ($t = 4.190$, $df = 7$, $P = 0.0041$) y 5HIAA ($t = 4.069$, $df = 7$, $P = 0.0048$) comparado con el grupo vehículo. Por otro lado, se verificó la posición de los electrodos en cada región, y fueron descartadas dos ratas del grupo vehículo y dos ratas del grupo 5HT-D por no cumplir con la correcta posición de electrodos en los sitios de registro. En la Figura 15A se representa la posición de los electrodos en el hipocampo y núcleos mamilares para las diferentes ratas.

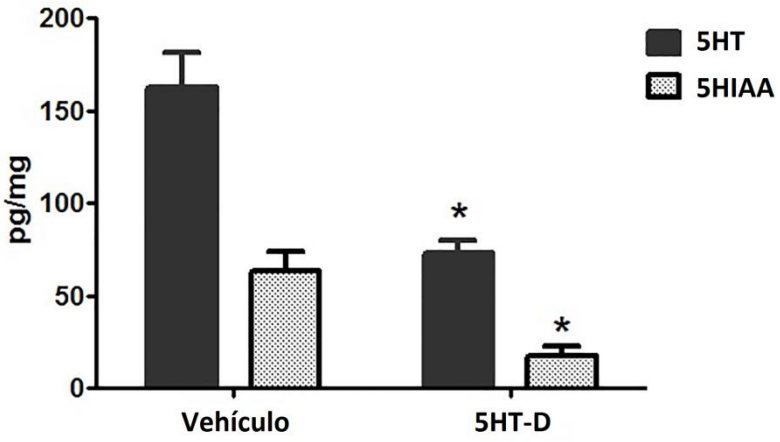


Figura 11. Concentración de la serotonina (5HT) y Acido 5-Hidroxi-indol acético (5HIAA) del septo medial. (pg/mg de tejido fresco). Media ± EEM. *, 5HT-D vs. Vehículo.

7.2 Conducta

La latencia de escape fue comparada a través de los días de entrenamiento en cada grupo. La Figura 12A muestra que ambos grupos tuvieron una reducción significativa en la latencia de escape a través de los días 2 al 6 con respecto al día 1: grupo vehículo (χ^2_r = 28.357 P = 0.0001) en el día 2 (p = 0.025) and los días 3-6 (p = 0.012); y el grupo 5HT-D (χ^2_r = 28.857 P = 0.001) los días 2-6 (0.012). En el análisis inter-grupal se obtuvieron diferencias significativas entre grupo ($\sum R_x$= 1245 P = 0.017), los días 2 (p = 0.001), 3 (p = 0.036) y tendencia en el día 1 (p = 0.058). Figura 13ª.

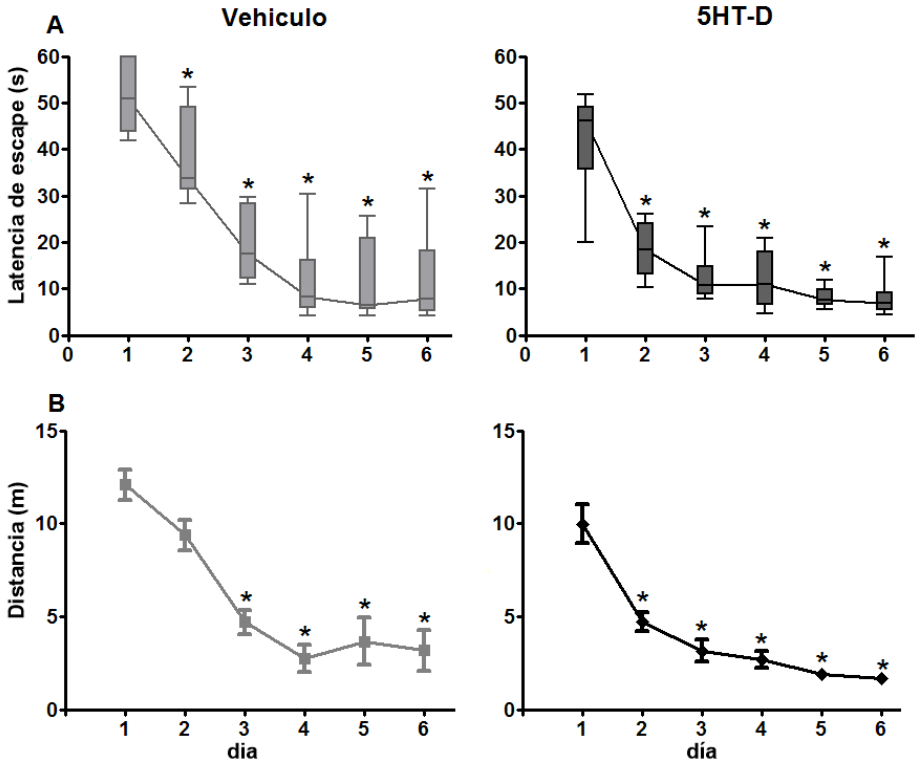


Figura 12. Comparación intra-grupal de las diferentes variables de conducta. (A) Latencia de escape en cada día de entrenamiento. Media ± E.E.M. (B) Distancia recorrida durante los días de entrenamiento Media ± E.E.M. *, día 1vs. subsecuentes días en el grupo vehículo y 5HT-D.

En la comparación intragrupal de la distancia se observó que el grupo vehículo ($F_{5,35}$ = 25.45, P < 0.0001) redujo la distancia recorrida a partir del día 3 (p < 0.01) hasta el 6 (p < 0.001, día 4 al 6) con respecto al día 1. Sin embargo, es remarcable que el grupo 5HT-D redujo significativamente sus distancias ($F_{5,35}$ = 38.59, P < 0.0001) un

día antes que el grupo vehículo, es decir, a partir del día 2 hasta el 6 ($p < 0.05$ todos los días) y comenzó con distancias más cortas desde el primer día. (Figura 12B). En el análisis inter-grupal se observó una interacción significativa del grupo y día ($F_{5,70} = 2.708$, $P = 0.0270$), donde el grupo 5HT-D recorrió significativamente distancias más cortas el día 2 ($p < 0.001$) respecto al grupo vehículo (Figura 13B).

Para la velocidad de nado se aplicó un análisis similar a la distancia y no se observaron cambios en ningún grupo por día de entrenamiento, como se muestra en la Figura 13D para el grupo vehículo ($F_{5,35} = 0.3646$, $P = 0.8693$), o para el grupo 5HT-D ($F_{5,35} = 0.5348$, $P = 0.7485$). La comparación inter-grupal no mostró diferencias significativas entre grupos ($F_{5,70} = 0.5403$, $P = 0.7451$).

En el análisis del número de cruces dentro del anillo central en cada cuadrante (correspondiente a la posición de la plataforma), se obtuvo una tendencia en la interacción de los factores ($F_{3,42} = 2.698$, $P = 0.057$), y como se muestra en la Figura 13C, el grupo 5HT-D obtuvo de forma significativa un mayor número de cruces dentro del anillo de la plataforma en el cuadrante norte ($p < 0.05$) respecto al grupo vehículo.

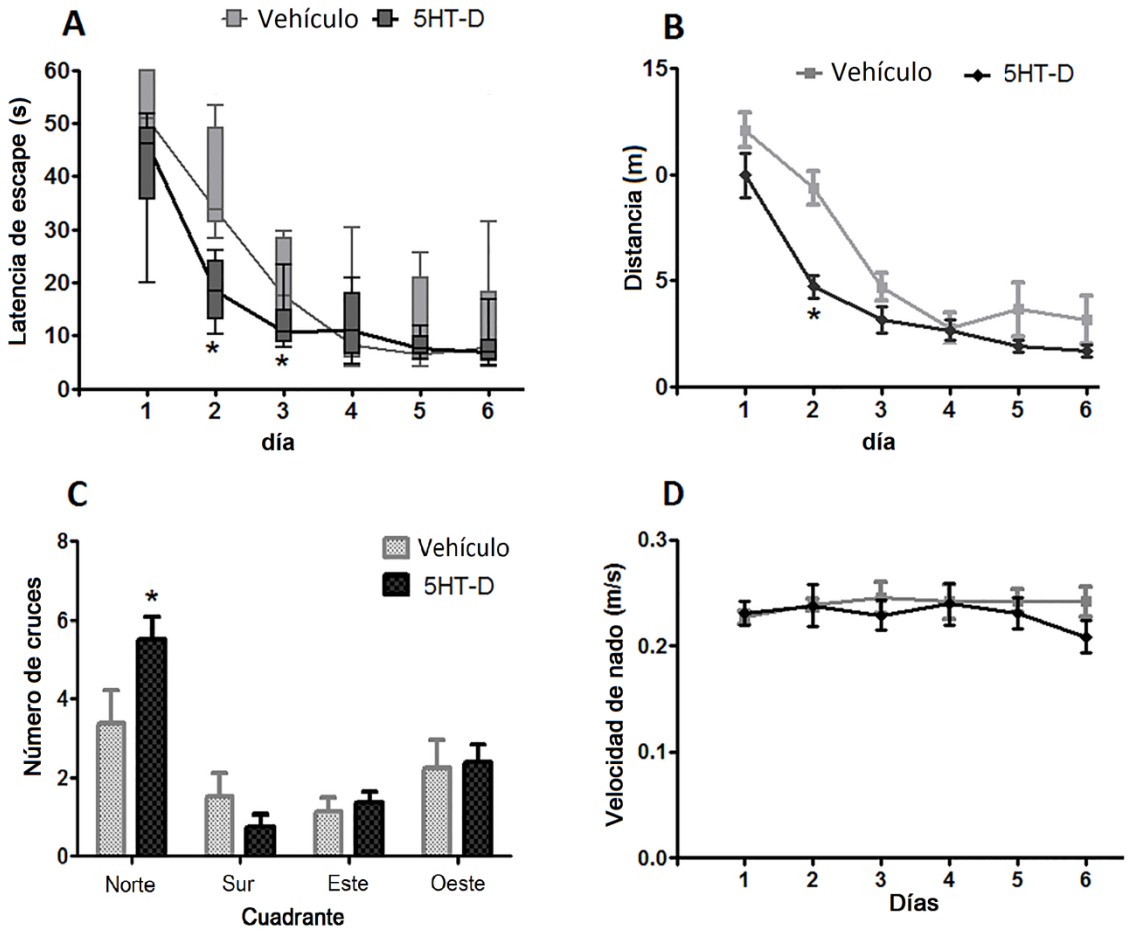


Figura 13. Comparación inter-grupo del aprendizaje en el laberinto acuático de Morris. (A) Latencia de escape en cada día de entrenamiento. Media ± E.E.M. (B) Distancia recorrida durante los días de entrenamiento Media ± E.E.M. *, 5HT-D vs. Vehículo. (C) Prueba de transferencia: Número de cruces dentro del anillo central en cada cuadrante (correspondiente a la posición de la plataforma). Media ± E.E.M. *, 5HT-D vs. Vehículo. (D) Velocidad de nado de los dos grupos. Media ± E.E.M.

7.3 Actividad EEG (potencia de la actividad theta)

Se analizó el EEG registrado en las diferentes regiones durante condición basal y durante la búsqueda de la plataforma en el laberinto acuático de Morris cada día de entrenamiento. En la Figura 15 se muestra un trazo representativo del EEG de diferentes sitios de registro, así como un esquema de la posición de los electrodos en cada sitio de registro para las diferentes ratas en cada grupo. En la Figura 14 se muestra un espectrograma de la región CA1 de una rata del grupo vehículo y una

del grupo 5HT-D, durante la condición basal y búsqueda de la plataforma (nado) del día 2 de entrenamiento. Se puede observar que durante la búsqueda de la plataforma predomina la actividad theta (potencia pico aproximadamente en 7.5 Hz) en ambos grupos y durante la condición basal predomina actividad irregular.

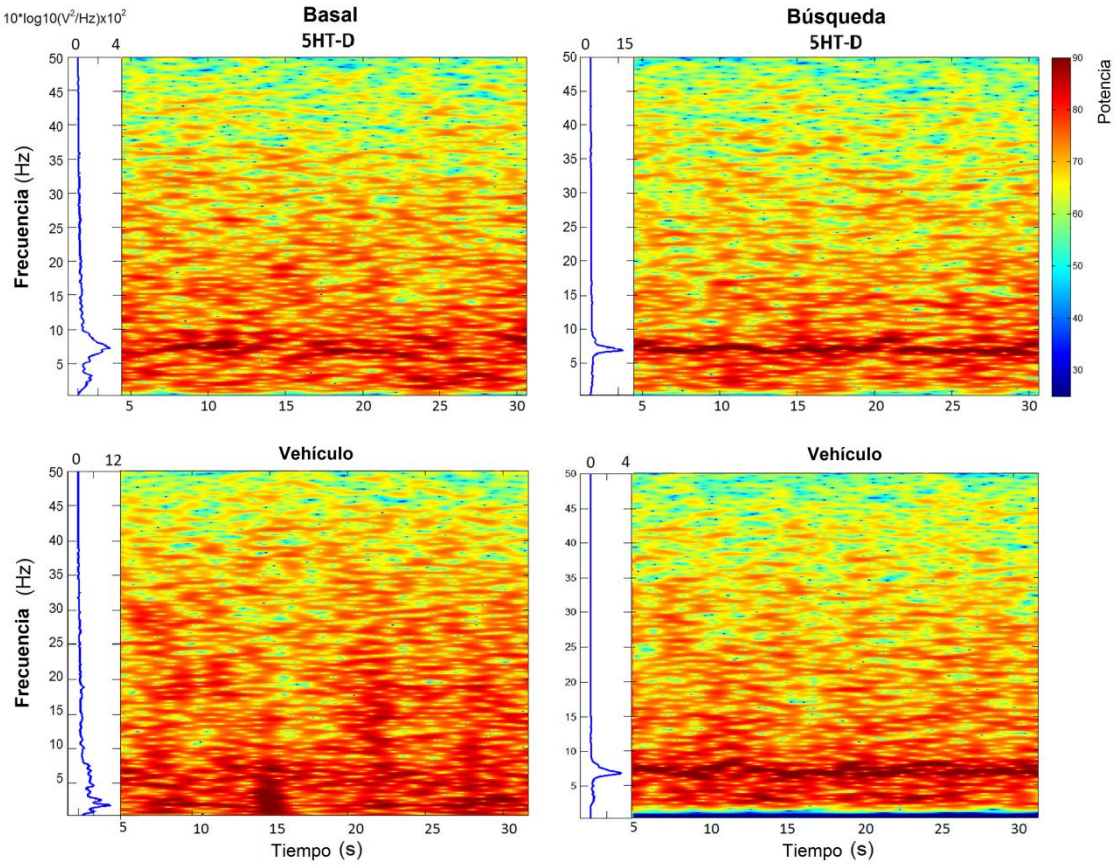


Figura 14. Espectrograma tiempo- frecuencia del EEG de una rata representativa durante la condición basal y búsqueda de la plataforma en cada grupo.

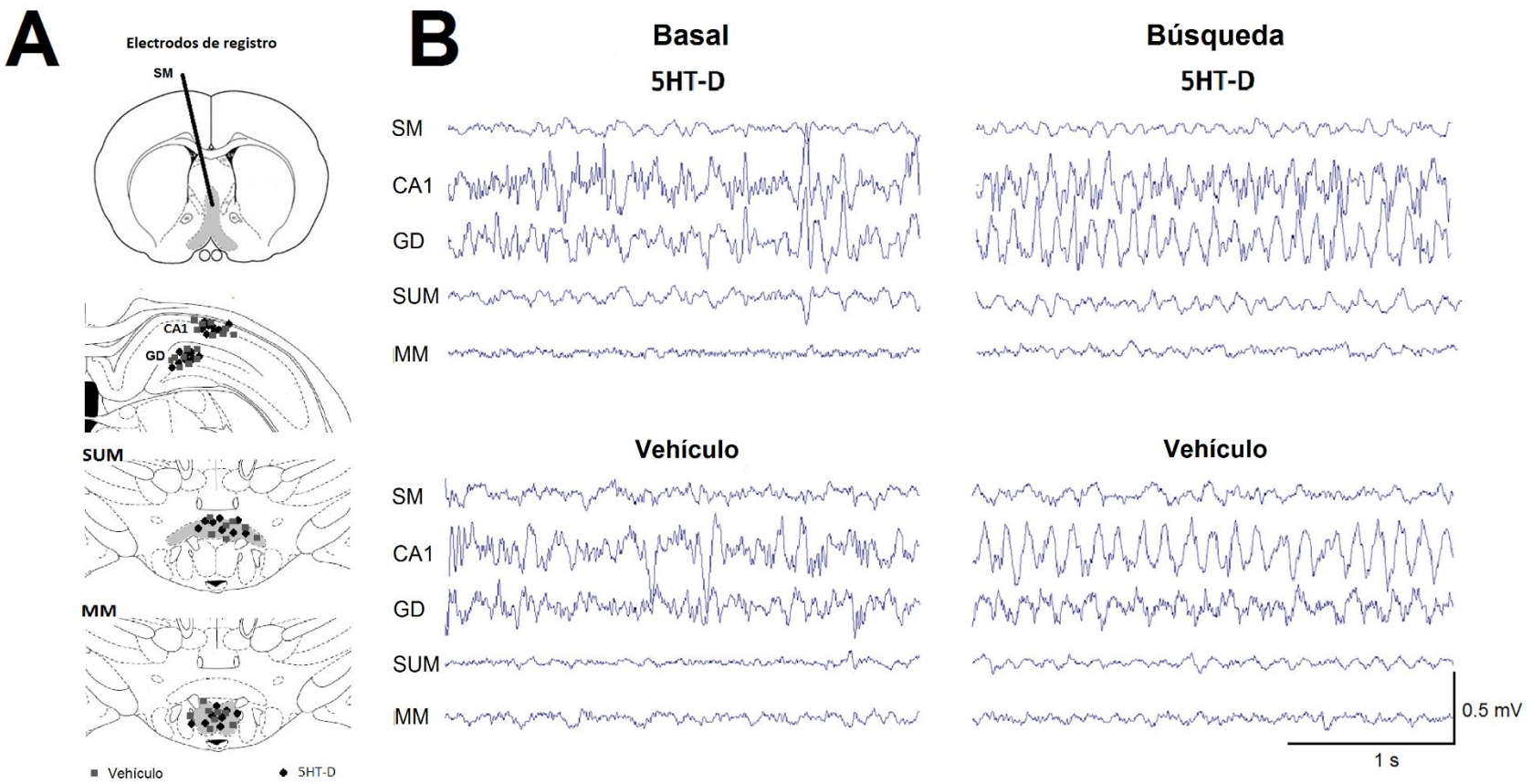


Figura 15. (A) Posición de los electrodos en cada sitio de registro para las diferentes ratas en cada grupo (B) Trazos EEG de una rata representativa de cada grupo. Muestra de 3 segundos durante condición basal y búsqueda de la plataforma. Cal. 0.5 mV/1 s. SM, septo medial; GD, giro dentado; CA1, cuerno de Ammon; SUM, núcleo supramamilar; MM, núcleo mamilar medial.

7.3.1 Giro dentado y CA1. En la Figura 16 se muestra la comparación intra-grupo de la PR del grupo vehículo, donde se observan cambios similares en la PR del GD y CA1 a través de los días de entrenamiento en el laberinto acuático de Morris (DG, $F_{80,510} = 4.163$, $P < 0.0001$; CA1, $F_{80,510} = 6.685$, $P < 0.0001$). En el grupo Vehículo Se observó un incremento significativo en la potencia de alta frecuencia (7.5-8-8.5 Hz), en los días 3 al 6 con respecto al día 1, mientras que las bajas frecuencias (6-6.5 Hz) disminuyeron con respecto al día 1. De igual manera, en el grupo 5HT-D la PR mostró cambios similares en el GD y CA1 durante los días de entrenamiento (GD, $F_{80,595} = 6.069$, $P < 0.0001$; CA1, $F_{80,595} = 4.765$, $P < 0.0001$); sin embargo, la PR de la actividad theta incrementó en las frecuencias 7.5 y 8 Hz desde el día 2, y las frecuencias bajas (6-6.5 Hz) disminuyeron con respecto al día 1. Es decir, los cambios observados en el grupo vehículo durante el día 3, se observaron un día antes (en el día 2) en el grupo 5HT-D, y la potencia y frecuencia de la actividad theta continuaron incrementando (a 8-8.5-9 Hz) durante los días 3 al 6, mientras que las bajas frecuencias (6-7 Hz) significativamente disminuyeron con respecto al día 1.

En general ambos grupos tuvieron bajas frecuencias en el día 1 y conforme avanzaron los días de entrenamiento, se incrementó la potencia y frecuencia las cuales se asociaron con la mayor eficiencia observada en la conducta, particularmente en la distancia recorrida. Los valores de las diferencias significativas en la PR durante los días de entrenamiento y regiones registradas se muestran en la Tabla1. En la Figura 16, se muestra la PR de GD y CA1 de los primeros 3 días (principales diferencias en la conducta) y en el día 6 (cuando el aprendizaje ya fue establecido; los resultados de los días 4 y 5 fueron similares al día 6).

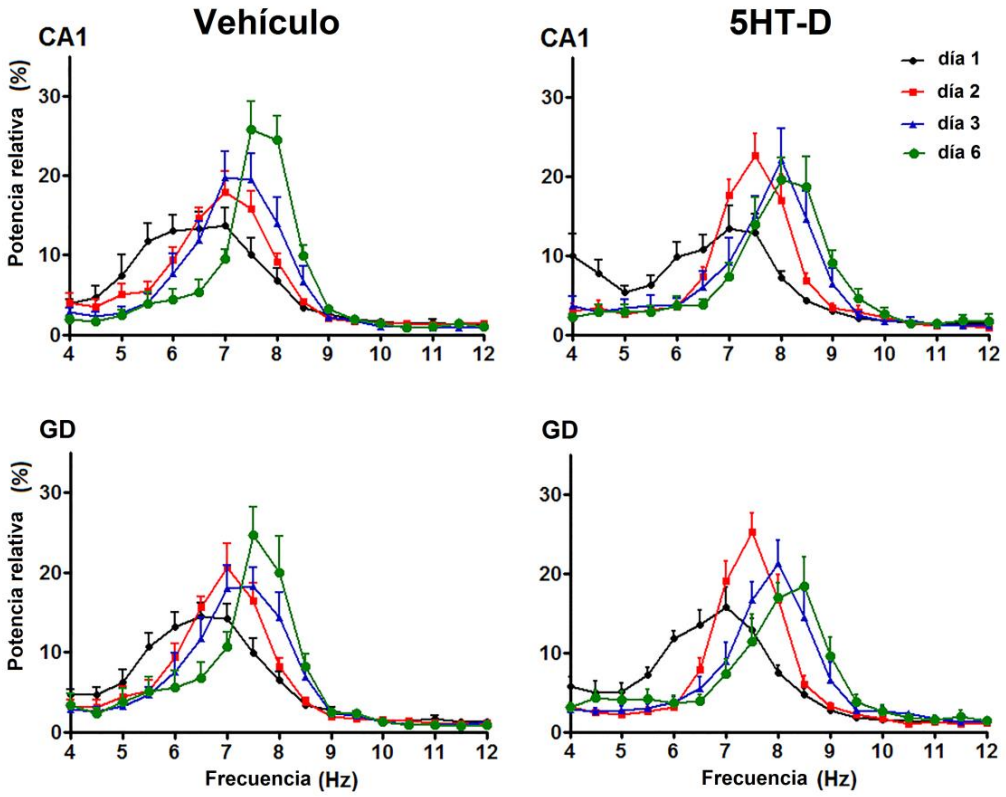


Figura 16. Potencia relativa de la actividad theta (4-12 Hz) registrada en el GD y CA1 en los grupos vehículo y 5HT-D durante la búsqueda de la plataforma cada día de entrenamiento. Solo los días 1, 2, 3 y 6 son mostrados. Media ± E.E.M. Las diferencias significativas se muestran en la Tabla 1, e incluye a los otros sitios de registro. $p < 0.05$.

Tabla 1. Cambios significativos de la PR en cada grupo

Región/ día	D1vsD2	D1vsD3	D1vsD4	D1vsD5	D1vsD6
SM Vehículo	ns	ns	ns	Ns	8
SM 5HT-D	/ 7.5-8	ns	ns	ns	Ns
GD Vehículo	ns	/ 7.5-8	5.5-6.5 / 7.5-8.5	5.5-6.5 / 7.5-8	5.5-6.5 / 7.5-8
GD 5HT-D	6-6.5 / 7.5-8	6-7 / 8-8.5	6-7 / 8-9	6-7 / 8-8.5	6-7 / 8-9
CA1 Vehículo	5.5 /	5.5 / 7-8	5.5-6.5 / 7.5-8.5	5.5-6.5 / 7.5-8	5.5-6.5 / 7.5-8.5
CA1 5HT-D	6 / 7.5-8	6 / 8-8.5	6-7 / 8-8.5	6-7 / 8-8.5	6-7 / 8-9
SUM Vehículo	ns	ns	5.5-6.5 / 7.5-8.5	5.5-6.5 / 7.5-8	5.5-6 / 7.5-8.5
SUM 5HT-D	/ 7.5-8	/8-8.5	/ 8-8.5	/ 8-8.5	/8-9
MM Vehículo	ns	5.5 / 7.5	5.5-6 / 7.5-8	5.5-6 / 7.5-8	5 / 7.5-8
MM 5HT-D	/7.5-8	/ 8	/ 8	/ 8-8.5	/ 8-8.5

Comparación intra-grupo de PR de los diferentes días de entrenamiento. Día 1 y los otros días de entrenamiento (D1vs D2 al D6). Valores antes de la diagonal- reduce de forma significativa; después de diagonal- incrementa de forma significativa. Valores de frecuencia en Hz. ns, no significativo. $p < 0.05$

Se realizó un análisis global de la PR de todos los 6 días de entrenamiento (promedio de la PR en cada frecuencia theta, en cada día) de los dos grupos. Es notable que en el grupo 5HT-D se observa una mayor frecuencia (8.5 Hz) pico en las regiones GD, CA1 y SUM con respecto al grupo vehículo durante la prueba del laberinto acuático de Morris (GD, $F_{16,85} = 3.890$, $P < 0.0001$; CA1, $F_{16,85} = 4.947$, $P < 0.0001$; SUM, $F_{16,85} = 5.176$, $P < 0.0001$). (Figura 17, antes de la flecha negra).

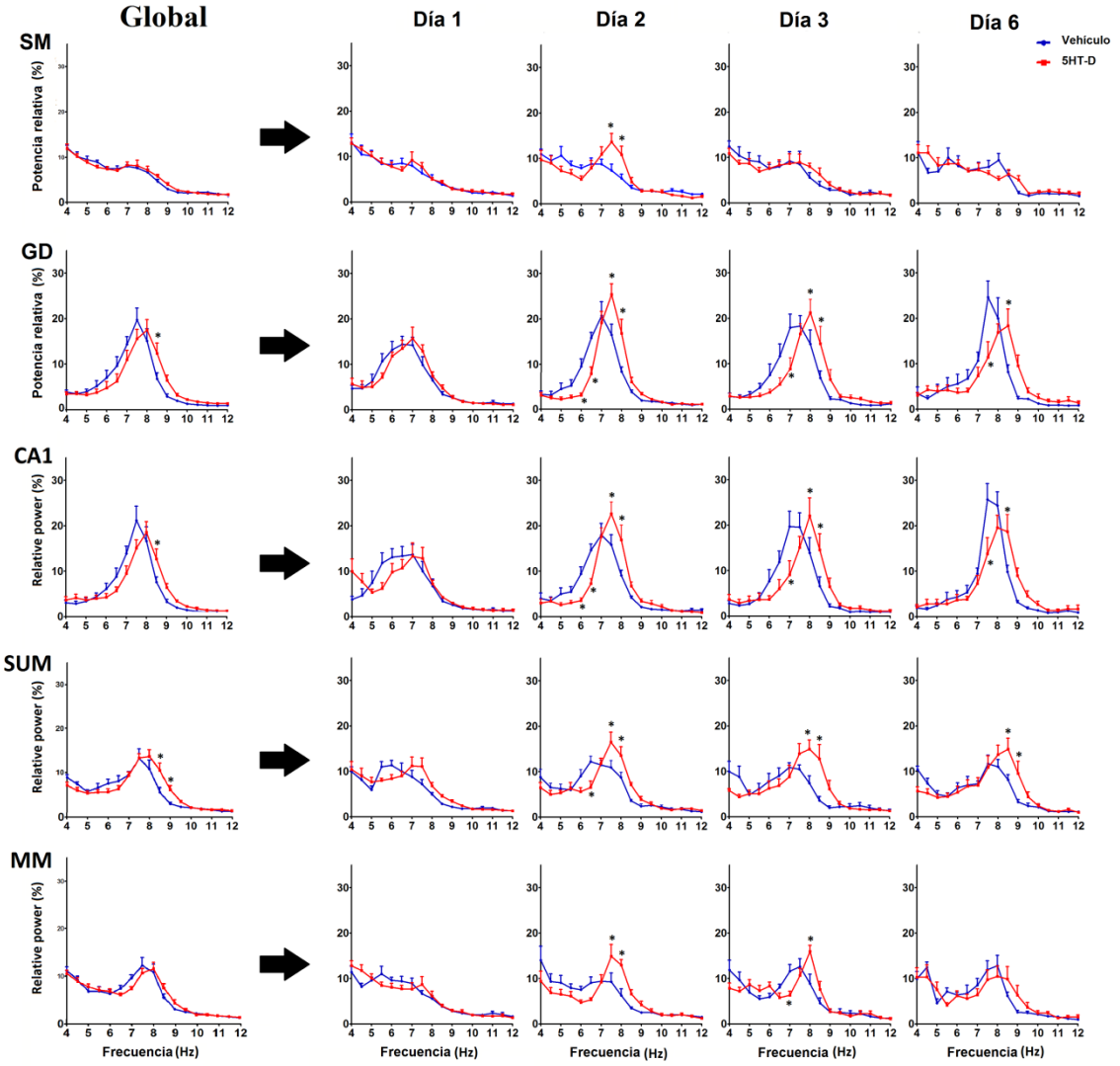


Figura 17. Izquierda: Potencia relativa y frecuencia de la actividad theta (4-12 Hz) en las diferentes regiones, durante el desempeño global (principal efecto) de todos los días en cada grupo. Derecha (después de la flecha), Potencia relativa de la actividad theta registrada durante la búsqueda de la plataforma en cada día. Solo son mostrados los días 1, 2, 3 y 6. Media ± E.E.M. *, 5HT-D vs Vehículo. $p < 0.05$.

Se realizó el análisis de correlación de Pearson de la frecuencia pico theta y la distancia recorrida o la velocidad en días de entrenamiento en cada rata. El grupo vehículo mostró una correlación negativa entre la distancia y la frecuencia pico theta de las regiones GD ($r = -0.3999$, $P = 0.0087$) y CA1 ($r = -0.5771$, $P = 0.0001$) del hipocampo (Figura 18A). Se observaron cambios similares en la correlación de las

mismas variables en el grupo 5HT-D en DG ($r = -0.5803$, $P = 0.0001$) y CA1 ($r = -0.5679$, $P = 0.0001$). Sin embargo, en la correlación de la frecuencia pico y velocidad de nado (Figura 18B), los grupos vehículo y 5HT-D no mostraron una correlación significativa a través de los días de entrenamiento en las regiones del GD (vehículo: $r = 0.0427$, $P = 0.7880$ and 5HT-D: $r = 0.0339$, $P = 0.8186$) y CA1 (vehículo: $r = 0.2652$, $P = 0.0897$; 5HT-D: $r = 0.0230$, $P = 0.8766$).

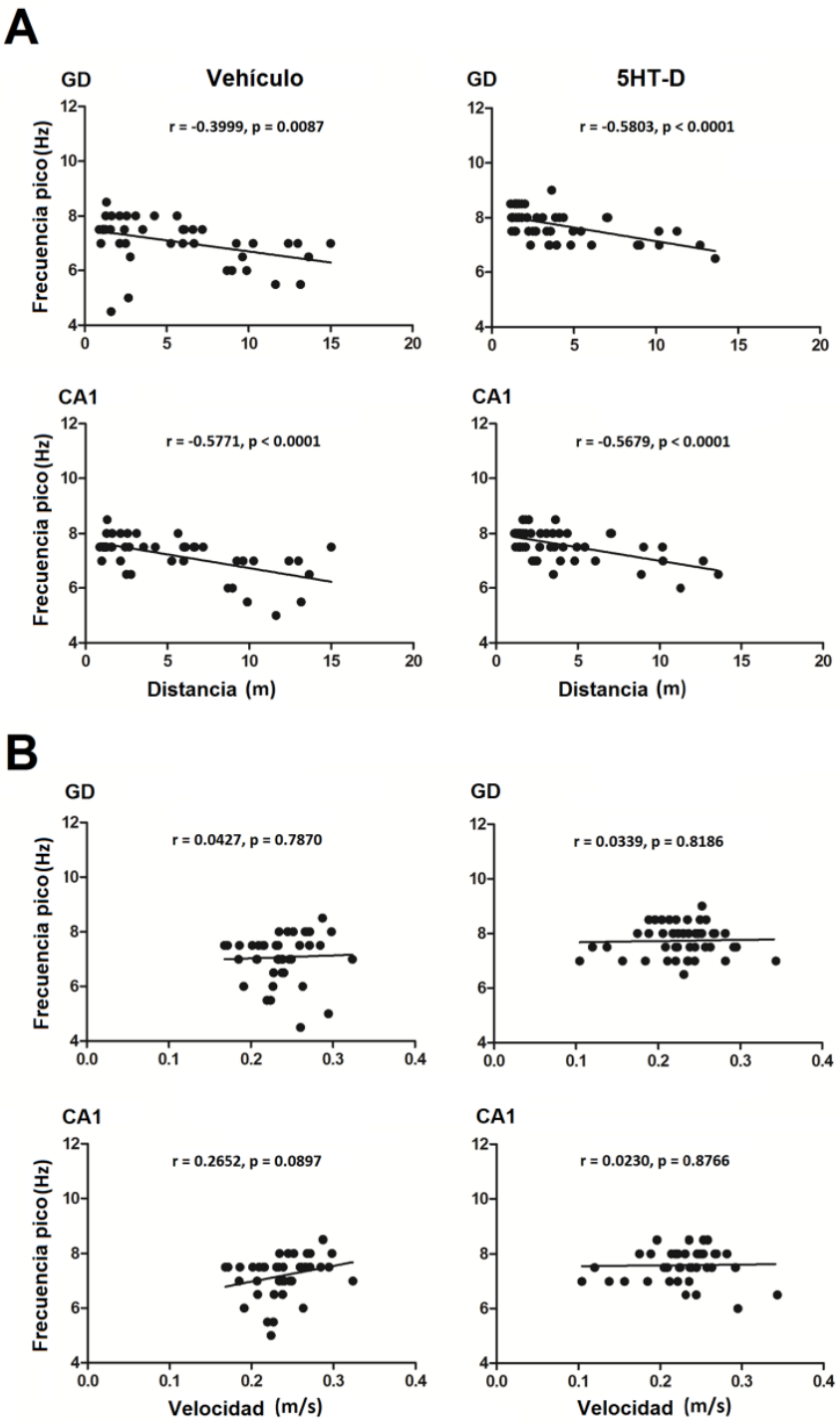


Figura 18. Correlación de Pearson entre la frecuencia pico theta y distancia o velocidad en ambos grupos. (A) Correlación entre la frecuencia pico theta y distancia en DG y CA1 del hipocampo. (B) Correlación de la velocidad con la frecuencia pico de GD y CA1. Ordenadas: frecuencia pico (Hz), abscisas: distancia (metros), velocidad (metros/segundo).

7.3.2 Septo medial. En el grupo vehículo, la PR no mostró significancia en la interacción de la frecuencia a través de los días ($F_{80,510}$ = 1.232, P < 0.0972); sin embargo, al realizar las comparaciones pareadas se mostró un incremento significativo de la potencia en la frecuencia de 8 Hz en el día 6. En el grupo 5HT-D se observó un efecto significativo en la interacción de la frecuencia y los días de entrenamiento ($F_{80,595}$ = 2.153, P < 0.0001) con un incremento en la PR en la frecuencia (7.5-8 Hz) solo durante el día 2 (Tabla 1).

7.3.3 Los núcleos supramamilar y mamilar medial (SUM-MM). El grupo vehículo mostro cambios significativos en la PR cuando se comparó a la frecuencia a través de los días (SUM, $F_{80,510}$ = 5.706, P < 0.0001; MM, $F_{80,510}$ = 3.588, P < 0.0001). Particularmente en los días 4, 5 y 6 la PR de alta frecuencia incrementó (7.5-8.5 Hz) y la PR en bajas frecuencias (5.5-6.5 Hz) disminuyó con respecto al día 1. En el grupo 5HT-D, la PR mostró cambios significativos en la PR a través de los días (SUM, $F_{80,595}$=3.018, P < 0.0001; MM, $F_{80,595}$ = 2.967, P < 0.0001), pero en contraste al grupo vehículo, la PR de alta frecuencia (7.5-8 Hz) incrementó desde el día 2 con respecto al día 1, y la PR de alta frecuencia (8-8.5-9 Hz) incremento con respecto al día 1 en los otros días de entrenamiento como se muestra en la tabla 1. Junto con los cambios observados en la PR de GD y CA1, el significado posible de estos resultados es que la reducción de serotonina del septo medial facilitó el incremento en la PR de alta frecuencia en las diferentes regiones, principalmente en el día 2. Estas diferencias son identificadas en la tabla 1 y en la comparación inter-grupal de la Figura 17. Las principales diferencias ocurren a frecuencias de 7.5-8 Hz en los días 2 y 3.

El análisis inter-grupal mostro un efecto significativo en la interacción de los factores frecuencia, grupo y día en la PR del hipocampo (DG, $F_{80,1105}$ = 2.599, P < 0.0001; CA1, $F_{80,1105}$ = 2.861, P < 0.0001) y núcleos mamilares (SUM, $F_{80,1105}$ = 1.853, P < 0.0001; MM, $F_{80,1105}$ = 3.102, P < 0.0001). Las comparaciones pareadas mostraron diferencias en las frecuencias 7.5-8 Hz durante los días 2, 3, y 6 (p < 0.001) en las diferentes regiones. Se encontró una tendencia en la interacción de los mismos factores en la PR del SM ($F_{80,1105}$ = 1.253, P = 0.061) y en la comparación pareada

se observaron diferencias en las frecuencias 7.5 y 8 Hz del día 2 ($p < 0.01$) (Figura 17, después de la flecha negra).

Al analizar la condición basal no se observaron diferencias en la PR de los grupos a través de los días de entrenamiento en la regiones de SM (Vehículo, $F_{80, 544}$ =0.3909, P = 1.000; 5HT-D, $F_{80, 640}$ = 0.7963, P = 0.8997), GD (Vehículo, $F_{80, 544}$ = 0.5552, P = 0.9993; 5HT-D, $F_{80, 640}$ = 0.7158, P = 0.9688), CA1 (Vehículo, $F_{80, 544}$ = 0.6167, P = 0.9958; 5HT-D, ($F_{80, 640}$ = 0.4836, P = 1.000), SUM (Vehículo, $F_{80, 544}$ = 0.7117, P = 0.9700; 5HT-D, $F_{80, 640}$ = 0.5313, P = 0.9997) and MM (Vehículo, $F_{80, 544}$ = 0.8077, P = 0.8819; 5HT-D, $F_{80, 640}$ = 0.8635, P = 0.7922). En la comparación inter-grupal se mostró un efecto significativo en la interacción de los factores grupo, frecuencia y día solo en el GD ($F_{(80,1105)}$ = 2.861, P <0.0001) del hipocampo: en el grupo 5HT-D se observó una menor PR en las frecuencias 6.5 y 7 Hz, comparada con el grupo vehículo.

7.4 Coherencia de la actividad theta

El análisis intra-grupo de la coherencia del EEG entre el SM-GD del grupo vehículo mostró significancia en la interacción de frecuencia y día ($F_{80,576}$ = 1.354, P = 0.0298). Las comparaciones pareadas mostraron una mayor coherencia en las frecuencias de 9.5-10.5 Hz ($p < 0.05$) solo en el día 6 con respecto al día 1. La coherencia del EEG entre SM-CA1también mostró significancia en la interacción frecuencia y día ($F_{80,576}$ = 1.633, P = 0.0070), y se observó un incremento en la coherencia en las frecuencias 8.5-9 Hz en el día 4 ($p < 0.001$), 7.5-9 Hz en el día 5 ($p < 0.001$) y 8-10 Hz en el día 6 ($p < 0.05$), con respecto al día 1. La coherencia del EEG entre SM-SUM no mostro significancia en la frecuencia través de los días ($F_{80, 576}$ = 1.220, p = 0.1824), sin embargo, en la comparación pareada mostro un incremento en la coherencia de las frecuencias 7.7-9.5 Hz ($p <0.05$) en los días 4, 5 y 6 ($p < 0.05$). Finalmente, la coherencia entre SM-MM tuvo significancia en la interacción de frecuencia y día ($F_{80,576}$ = 1.344, P = 0.0482), con un incremento de coherencia en las frecuencias 8-9 Hz durante los días 4, 5 y 6 con respecto al día 1 ($p < 0.05$).

En la comparación de la coherencia del EEG del grupo 5HT-D se observó un efecto significativo en la interacción de factores (frecuencia, día) SM-GD ($F_{80,672} = 1.483$, $P = 0.0061$). La comparación pareada muestra un incremento en la coherencia del día 2 en la frecuencia de 7.5 Hz ($p < 0.05$) y en el día 6 a las frecuencias de 8 y 9 Hz ($p < 0.05$) con respecto al día 1. La coherencia del EEG entre SM-CA1 ($F_{80, 672} = 1.359$, $P = 0.0262$) fue mayor en el día 2 en la frecuencia de 7.5 Hz ($p < 0.05$) y en el día 6 en las frecuencias 8 y 9.5 Hz ($p < 0.05$) con respecto al día 1. La coherencia del EEG entre SM-SUM ($F_{80,672} = 1.320$, $P = 0.0402$) mostro efecto significativo sobre los días de entrenamiento, sin embargo, las comparaciones pareadas no mostraron cambios significativos. La coherencia del EEG entre SM-MM ($F_{80,672} = 1.474$, $P = 0.0069$) incremento en las frecuencias 8-8.5 Hz en el día 6 ($p < 0.05$) con respecto al día 1. Estos datos se encuentran resumidos en la Tabla 2.

Tabla 2. Coherencia del septo medial-hipocampo y septo medial-núcleos mamilares

Región/ día	D1vsD2	D1vsD3	D1vsD4	D1vsD5	D1vsD6
Vehículo SM-GD	ns	Ns	ns	ns	9.5-10.5
5HT-D	7.5	ns	ns	ns	8 y 9
Vehículo SM-CA1	ns	Ns	8.5-9	7.5-9	8-10
5HT-D	7.5	ns	ns	ns	8 y 9.5
Vehículo SM-SUM	ns	Ns	8-9.5	8-9.5	8
5HT-D	ns	ns	ns	ns	ns
Vehículo SM-MM	ns	Ns	8-9	8 and 9	8 y 9
5HT-D	ns	ns	ns	ns	8 and 8.5

Comparación intra-grupo entre el día 1 y los otros días de entrenamiento (D1vs D2 a D6). Valores de frecuencia en Hz. ns, no significativo. $p < 0.05$

Para identificar un efecto global, se realizó un análisis de la coherencia y frecuencia de todos los 6 días de entrenamiento durante la búsqueda de la plataforma (promedio de coherencia y frecuencia theta de cada día) en cada grupo. Es interesante notar que el grupo 5HT-D presentó mayor coherencia en el EEG registrado de SM-GD ($F_{16,85} = 1.305$, $P = 0.02132$) y SM-SUM ($F_{16,85} = 1.504$, $P = 0.01171$) en las frecuencias de 7.5 y 8 Hz ($p < 0.05$) con respecto al grupo vehículo (Figura 19, antes de la flecha negra).

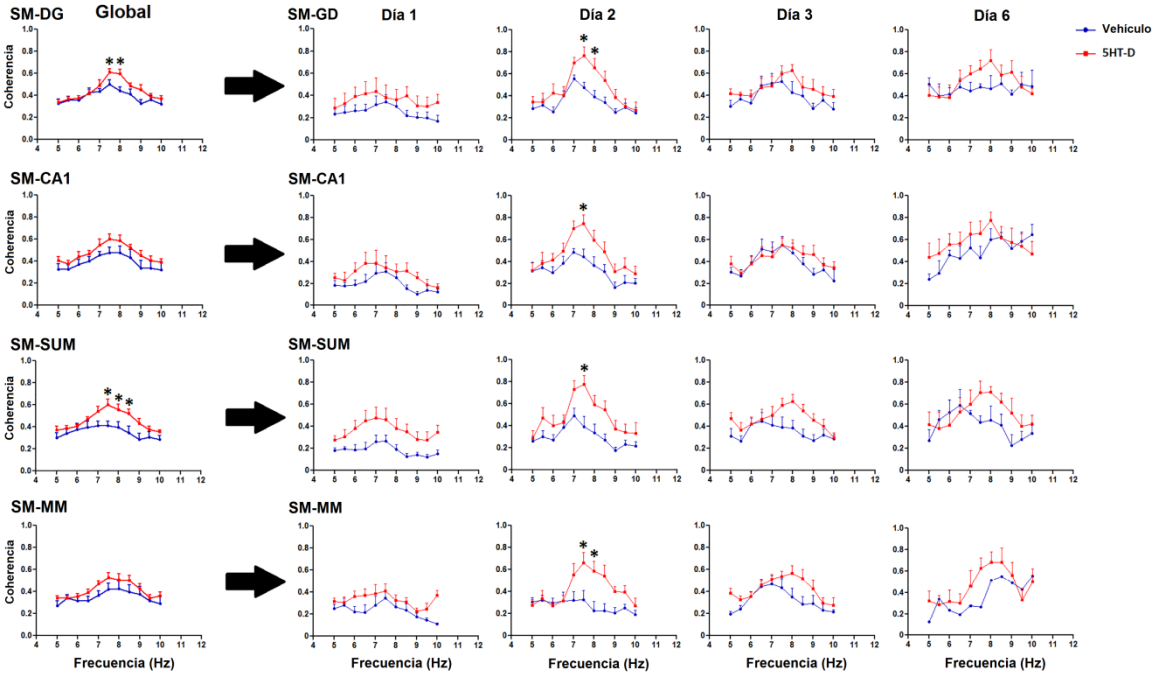


Figura 19. Comparación inter-grupo de la coherencia entre la actividad theta (4-12 Hz) registrada en SM-GD, SM-CA1, SM-SUM, SM-MM en cada día de entrenamiento. Izquierda: Coherencia durante el desempeño global de todos los días en cada grupo (efecto principal). Derecha (después de la flecha): Coherencia en las mismas regiones durante cada día de entrenamiento en la búsqueda de la plataforma. Solo se muestran los días 1,2, 3 y 6. Media ± E.E.M. *, 5HT-D vs Vehículo. $p < 0.05$.

En el análisis inter-grupal de la coherencia se observó que la coherencia entre SM-GD ($F_{80,2315} = 1.486$, $P = 0.045$) y SM-CA1 ($F_{80,2315} = 1.302$, $P = 0.039$) mostró efecto significativo en la interacción de frecuencia, grupo y día. Al realizar las comparaciones pareadas en el grupo 5HT-D se observó mayor coherencia en el día 2 en la frecuencia 7.5 y 8 Hz para SM-GD y en la frecuencia de 7.5 Hz para SM-CA1, con respecto al grupo vehículo. La coherencia SM-SUM mostró una tendencia en la interacción de los mismos factores ($F_{80,\ 2390} = 1.236$, $P = 0.079$), en la comparación pareada se mostró mayor coherencia en el grupo 5HT-D en la frecuencia de 7.5 Hz ($p < 0.01$) del día 2 ($p < 0.01$). De forma similar, la coherencia de SM-MM ($F_{80,2390} = 1.272$, $P = 0.068$) el grupo 5HT-D fue mayor durante el día 2 en la frecuencia 7.5-8.5 Hz ($p < 0.01$) comparado con el grupo vehículo (Figura 19, después de la flecha negra). La coherencia entre las regiones durante la condición

basal no mostro diferencia significativa por día y frecuencia en cada grupo y entre grupos (datos no mostrados).

B. Resultados del experimento 2: grupo CITAL y LCFA

Como ya se mencionó anteriormente, en este experimento solo se evaluó el aprendizaje y memoria espacial después de la aplicación de citalopram en el SM.

7. 4 Conducta

La latencia de escape fue comparada a través de los días de entrenamiento en cada grupo. En el grupo LCFA se observó una reducción significativa en la latencia de escape a través de los días 2 al 6 con respecto al día 1: grupo LCFA (χ^2_r = 32.492 P = 0.0001) en el día 2 (p = 0.021) and los días 3-6 (p = 0.008); mientras que en el grupo CITAL (χ^2_r = 34.851 P = 0.0001) se observó una reducción significativa a partir del día 3 (p = 0.036), hasta el resto de la prueba: día 4 (p = 0.037), 5 (p = 0.003) y 6 (p = 0.003) con respecto al día 1 (Figura 20A). En el análisis inter-grupal se obtuvieron diferencias significativas ($\sum R_x$= 841.5 P = 0.0001); el grupo CITAL presentó mayor latencia a partir del día 2 (p = 0.004), 3, 4, 5 (p = 0.003 para todos los días) y hasta el 6 (p = 0.014) de entrenamiento con respecto al grupo LCFA. (Figura 21A).

En la comparación intra-grupo de la distancia se observó que el grupo LCFA ($F_{5,40}$ = 9.348, P < 0.0001) redujo la distancia recorrida a partir del día 3 hasta el 6 (p < 0.05) con respecto al día 1. Sin embargo, el grupo CITAL redujo la distancia ($F_{5,50}$ = 4.28, P = 0.0026) hasta el día 6 (p < 0.05) con respecto al día (Figura 20B). En el análisis inter-grupal se observó una tendencia en la interacción del grupo y día ($F_{5,90}$ = 2.12, P = 0.0597), donde el grupo CITAL recorrió significativamente distancias más largas los días 4 y 5 (p < 0.05 los dos) respecto al grupo LCFA. (Figura 21B).

Para la velocidad de nado se aplicó un análisis similar a la distancia y no se observaron cambios en ningún grupo por día de entrenamiento, como se muestra en la Figura 21D para el grupo LCFA ($F_{5,40}$ = 2.193, P = 0.0841), o para el grupo

CITAL ($F_{5,50}$ =1.447, P = 0.2239). La comparación inter-grupal no mostró diferencias significativas entre grupos ($F_{5,90}$ = 0.3356, P = 0.8901).

En el análisis del número de cruces dentro del anillo central en cada cuadrante (correspondiente a la posición de la plataforma), se observó un efecto significativo ($F_{3,45}$ = 2.686, P = 0.0577), en el cual el grupo CITAL tuvo un menor número de cruces dentro del anillo de la plataforma en el cuadrante norte ($p < 0.05$) respecto al grupo LCFA. (Figura 21C).

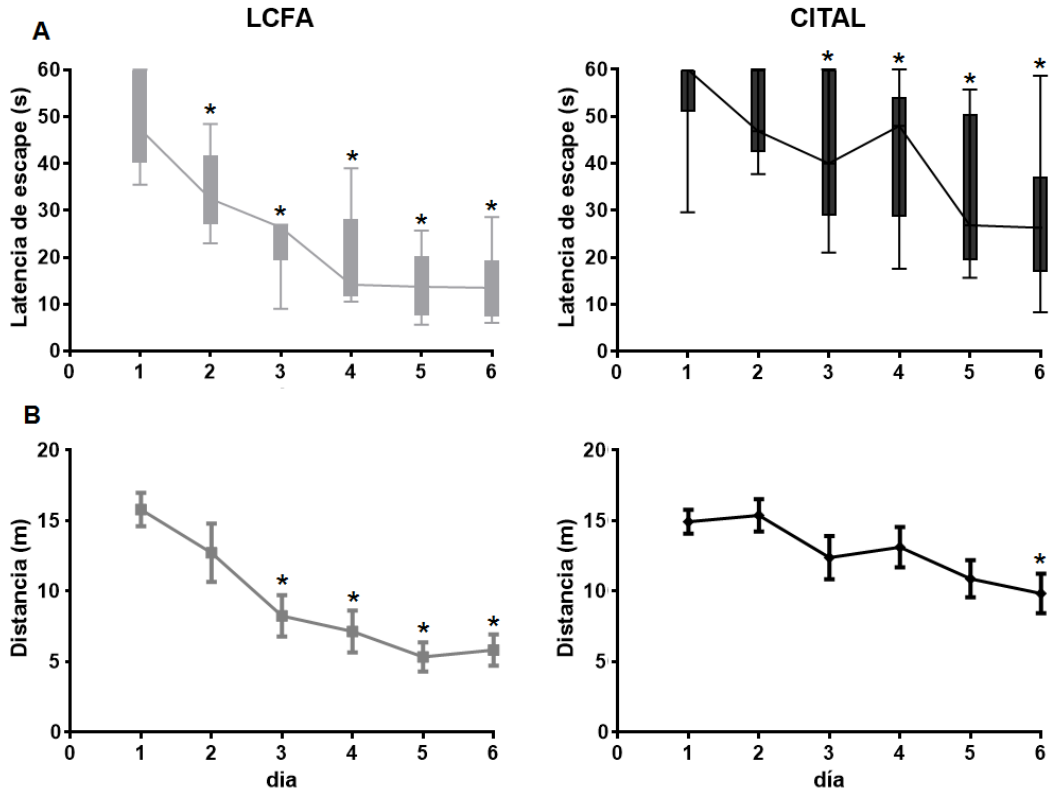


Figura 20. Comparación intra-grupo de las diferentes variables de conducta. (A) Latencia de escape en cada día de entrenamiento. Media ± E.E.M. (B) Distancia recorrida durante los días de entrenamiento Media ± E.E.M. *, día 1vs. subsecuentes días en el grupo CITAL y LCFA.

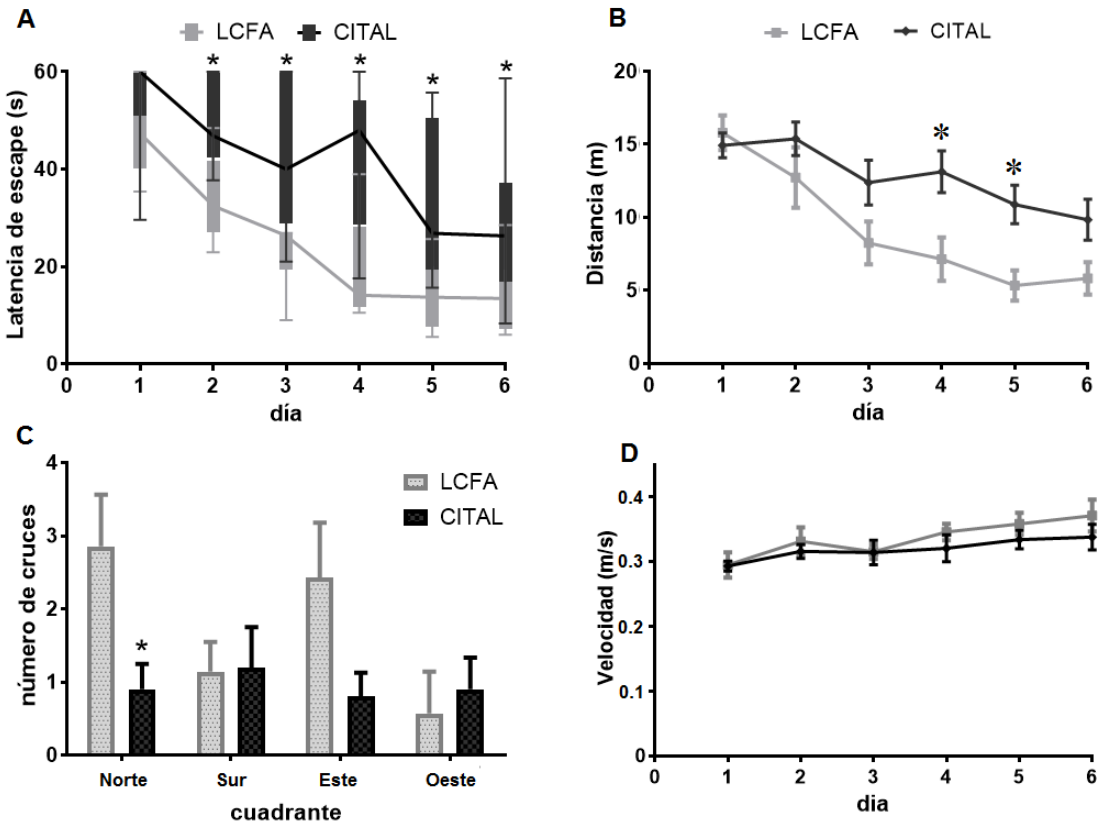


Figura 21. Comparación inter-grupo del aprendizaje en el laberinto acuático de Morris. (A) Latencia de escape en cada día de entrenamiento. Media ± E.E.M. (B) Distancia recorrida durante los días de entrenamiento Media ± E.E.M. (C) Prueba de transferencia: Número de cruces dentro del anillo central en cada cuadrante (correspondiente a la posición de la plataforma). Media ± E.E.M. (D) Velocidad de nado de los dos grupos. Media ± E.E.M. *, CITAL vs. LCFA

8. DISCUSIÓN

El papel funcional de la serotonina en el septo medial y su repercusión sobre la actividad theta que ocurre durante conductas que involucran la codificación y recuperación de la información como en el aprendizaje y la memoria espacial dependiente del hipocampo es poco comprendida. Los resultados del presente estudio demuestran que la reducción de la serotonina septal induce un mejor desempeño en el procesamiento inicial del aprendizaje. Así, el grupo 5HT-D mostró una reducción temprana en la distancia y latencia de escape, además de que iniciaron la prueba con distancia menor en el día 1 así como una mayor exactitud para encontrar la plataforma como se deduce del mayor número de cruces en el sitio preciso donde se encontraba la plataforma. En contraste, también se demostró que el aumento de la serotonina en el septo medial ocasionó en los animales una menor eficiencia para resolver la tarea, ya que a pesar de que reducen el tiempo y distancia a través de los días, la latencia y distancia de escape son considerablemente mayores con respecto a su grupo control.

Estos resultados sugieren que la reducción de serotonina septal favorece el procesamiento de información espacial con un efecto principal en la adquisición y codificación durante los primeros días de entrenamiento, así como en la recuperación de la información, mientras que un aumento en la serotonina septal afecta la codificación y recuperación de la información lo que está de acuerdo con el modelo de Jeltsch-David et al., (2008) que se retomará más adelante.

En apoyo a los resultados anteriores, ya se ha establecido que el SM juega un papel crítico en la regulación de la función hipocampal (Winson, 1979; Mamad et al., 2015; Tsanov, 2015). Se ha demostrado la participación septal en la adquisición y mantenimiento de la memoria espacial en el laberinto acuático de Morris, ya que la aspiración o lesión electrolítica del SM deteriora estos procesos (Hagan et al., 1988; Fraser et al., 1991; Brandner y Schenk., 1998; Decker et al., 1994). Además, la infusión intra-septal de lidocaína altera la adquisición y recuperación de la tarea en el laberinto acuático de Morris (validado en Koenig et al., 2008). Se sabe que la

importante participación del septo medial en los procesos de aprendizaje y memoria es mediado por las células GABAérgicas y colinérgicas (Nakagawa y Takashima, 1997; Nakagawa et al., 1995) Así, se tiene que la aplicación intra-septal de muscimol deteriora la adquisición de información espacial (Brioni et al., 1990) y deteriora la memoria a largo plazo, sugiriendo que las células GABAérgica septales tienen un papel importante en la regulación septo-hipocampal (Nagahara y McGaugh., 1992). También se ha demostrado el importante papel de las células colinérgicas septales en el aprendizaje espacial (Deiana et al., 2011; Hasselmo et al., 1995; Hasselmo y McGaughy, 2004). Ikonen et al., (2002) sugiere que la perdida de actividad colinérgica septo-hipocampal afecta el remapeo, importante para el establecimiento de la representación espacial del hipocampo en diferentes ambientes. Similarmente, Pang et al., (2001), mostraron que la lesión combinada de células GABAérgicas y colinérgicas septales produce el deterioro en la memoria espacial en el laberinto radial de 8 brazos y en el laberinto acuático de Morris, a pesar de que lesión separada a cada una de estas células no deteriora o deteriora medianamente la memoria espacial (Pan et al., 2001). Esto sugiere que los dos tipos de células son importantes para el aprendizaje espacial ya que su lesión produce profundos efectos sobre la fisiología hipocampal (Lee et al., 1994; Yoder y Pang, 2005).

La actividad de células septales GABAérgicas, colinérgicas y probablemente glutamatérgicas puede ser modulada por aferentes extrínsecos, particularmente por terminales serotoninérgicas que hacen sinapsis con neuronas del septo e hipocampo que expresan diferentes tipos de receptores serotoninérgicos (Milner y Veznedaroglu, 1993; Cassel y Jeltsch, 1995; Acsády et al., 1996), incluyendo 5HT1, 5HT2A, 5HT2C y otros receptores (Leranth y Vertes, 1999). La facilitación y afectación en el procesamiento de información espacial observada en el grupo 5HT-D y CITAL pudo deberse a la acción de la serotonina sobre estas células septales.

La participación de la serotonina en el aprendizaje espacial ha sido controversial, ya que en varios estudios se ha mostrado que la eliminación de serotonina produce una facilitación sobre el aprendizaje, principalmente en la adquisición de la tarea de aprendizaje en los laberintos Biel y Stone (Perez-Vega et al., 2000; Altman et al.,

1990; Normile et al., 1990), así como en evitación inhibitoria en el ratón (Altman et al., 1984). Y la eliminación cerebral de serotonina, no tiene efecto sobre el aprendizaje evaluado en los laberintos acuático de Morris y radial de 8 brazos (Murtha y Pappas, 1994). Sin embargo, se han observado deficiencias en el aprendizaje espacial causado por la lesión septal con 192 IgG-saporin (afecta las células colinérgicas), y el efecto es revertido por la eliminación de la serotonina hipocampal (Lehmann et al., 2002), lo cual sugiere que la atenuación del tono serotoninérgico en el hipocampo podría compensar algunas disfunciones subsecuentes a la perdida de entrada colinérgica hacia el hipocampo (Lehmann et al., 2002; Richter-Levin et al., 1993). Esta observación va de acuerdo con datos en los cuales se muestra que una reducción del tono serotoninérgico, por activación farmacológica de receptores somatodendriticos 5-HT1A de neuronas del rafé, atenúan la alteración cognitiva producida por la infusión intrahipocampal de drogas antimuscarínicas como la escopolamina (Carli et al., 1998, 2000). Además, la reducción de serotonina hipocampal da origen a una facilitación en el aprendizaje de lugar en el laberinto acuático (Gutiérrez-Guzmán et al., 2011). De forma similar, en el presente trabajo, la reducción de serotonina septal facilitó el aprendizaje de lugar a través de un efecto principal sobre la fase de adquisición de información y el aumento de la serotonina afecta el aprendizaje, lo cual sugiere que la serotonina en el septo medial juega un papel muy importante en la modulación de la codificación de información dependiente de la función hipocampal.

En contraste, cuando se aplica agonista (8-OH-DPAT) al receptor 5HT1A en el septo medial (Jeltsch et al., 2004; Bertrand et al., 2000), en el hipocampo (Carli et al., 1992) o cuando es administrado sistémicamente (Carli y Samanin, 1992), se deteriora el desempeño en el LAM y la memoria de trabajo. Específicamente, cuando el agonista intra-septal se administra pre-adquisición impide el aprendizaje en el LAM (Koenig et al., 2011). Durante esta tarea, la codificación de memoria espacial es alterada y se interfiere con la consolidación, pero no afecta la recuperación de la memoria (Koenig et al, 2008). Koening (2011), ha sugerido que el efecto de este receptor 5HT1A sobre la adquisición podría ser mediado por células no colinérgicas, como las GABAérgicas y glutamatérgicas o a través de

todos los tipos de células, ya que la administración intra-septal del agonista (8-OH-DPAT) combinado con una dosis subumbral de antagonistas al receptor NMDA (D-AP5) solo afecta (levemente) la adquisición espacial, mientras que deteriora profundamente la memoria espacial (Elvander-Tottie et al., 2009), sugiriendo la posible modulación serotoninérgica de las células glutamatérgicas. Además, la estimulación de los receptores 5HT1A y 5HT1B induce pobre desempeño en el aprendizaje espacial (Buhot et al., 1995; Carli et al., 1995).

Con base en la evidencia de los estudios anteriormente mencionados, la facilitación en la codificación de información después de la reducción de serotonina septal y el bajo rendimiento en el aprendizaje después del aumento de serotonina septal observado en este estudio podría ser mediado por receptores inhibidores localizados sobre células septales. Esto podría estar de acuerdo con la hipótesis de que la activación de neuronas colinérgicas y GABAérgicas es facilitada por un bajo tono serotoninérgico en el septo medial, favoreciendo la codificación y consolidación (Jeltsch-David et al., 2008). Por otro lado, este resultado podría ser explicado por el hecho de que altos niveles de Acetilcolina activan circuitos para facilitar la codificación de nueva información y bajos niveles de la misma podría no ser suficiente para la codificación de nueva información, pero facilitarían la consolidación y recuperación de información familiar (Hasselmo et al., 1995; Hasselmo y McGaughy, 2004).

Por otro lado, los cambios observados en la conducta del grupo 5HT-D se han asociado con cambios en la potencia relativa y coherencia a frecuencias especificas (7.5-8 Hz) de actividad theta en el hipocampo, y núcleos SUM y MM, durante los primeros días de aprendizaje. Conforme los animales alcanzan un aprendizaje significativo o éste es establecido, la potencia y frecuencia incrementa, es decir ocurre un cambio a frecuencias rápidas (7.5-8 Hz) de la actividad theta hipocampal (Gutiérrez Guzmán et al., 2012; Hernández-Pérez et al., 2015). Un afecto similar fue observado en el grupo Vehículo donde la PR de las frecuencias rápidas de la actividad theta del hipocampo (GD y CA1) fue asociada con reducción en la latencia

y distancia recorrida durante los días de entrenamiento (desde el día 3). Sin embargo, mientras ambos grupos incrementan la frecuencia a través de los días de entrenamiento, es notable que el grupo 5HT-D tuvo una mayor frecuencia pico desde el día 1 (7.5-8 Hz), y el cambio a altas frecuencias ocurrió desde el día 2, y conforme los días avanzan la frecuencia pico incrementa a 8-9 Hz (fue mantenida hasta el último día de entrenamiento). Ha sido demostrado que cambios en la frecuencia theta hipocampal pueden ser funcionalmente significativos y una frecuencia especifica puede ser critica para el funcionamiento hipocampal (McNaughton et al., 2006), por ejemplo, se ha demostrado que una reducción de aproximadamente de 0.5 Hz está relacionada con un modesto deterioro en el aprendizaje espacial (Pan y McNaughton, 1997). Ha sido reportado que una reducción de la frecuencia theta hipocampal por novedad ambiental (foraging), pero la frecuencia theta incrementa con los días de experiencia en ese ambiente (Jeewajee et al., 2008). En el presente estudio se observó que cambios en frecuencias especificas están asociados con un mayor acoplamiento funcional entre el septo medial con el hipocampo y núcleos mamilares, lo cual fue reflejado en una mayor coherencia a través del aprendizaje en el grupo 5HT-D que ocurrió principalmente en el día 2. Estos estudios podrían indicar que la reducción de serotonina del septo medial facilita no solo la comunicación del septo medial con el hipocampo, sino también con los núcleos mamilares para facilitar la codificación de información, principalmente durante la fase inicial del aprendizaje, como se expresó en la conducta. Una sincronización de la actividad theta durante este momento podría ayudar a lograr un óptimo nivel de comunicación que es mantenido hasta los últimos días. Anatómicamente, se ha reportado la comunicación reciproca del septo medial con el hipocampo y núcleos mamilares (Gonzalo-Ruiz et al., 1992; Borhegyi y Freund, 1998; Leranth et al., 1999), así como conexiones colaterales del SUM con el hipocampo (Vertes y McKenna, 2000); sin embargo, en el presente estudio no es posible determinar cuál región está influyendo sobre la otra, es decir se desconoce si es a través de la influencia directa del septo medial o del hipocampo los que conducen los cambios en la potencia del SUM. Lo que sí es claro es que la óptima dinámica de comunicación en los circuitos septo-hipocampal y septo-mamilar para

una eficiente codificación, consolidación y recuperación de información puede ser modulada por la serotonina actuando sobre células septales.

Ha sido mostrado que la lesión del SM con ácido iboténico reduce la frecuencia de la actividad theta (Leung et al., 1994). Además, ha sido mostrado que el SUM es importante para codificar la frecuencia theta hipocampal en ratas anestesiadas y en libre movimiento (Woodnorth et al., 2003; Pan y McNaughton, 2004). Por su parte, el núcleo mamilar medial integra información descendente del hipocampo que envía hacia el SUM, es importante para la codificación de información espacial y la modulación de actividad theta de CA1 (Kocsis y Vertes, 1994, 1997; Vann y Aggleton, 2004). Ha sido mostrado que el bloqueo del septo medial con tetracaina no afecta la ritmisidad en el SUM (McNaughton et al., 2006); sin embargo, la actividad theta lenta espontanea en el SUM sigue a la actividad theta de origen septal en ratas anestesiadas (Kocsis, 2006; Kocsis y Kaminski, 2006). En el presente estudio, solo las fibras serotoninérgicas fueron eliminadas y a pesar de que no fueron observados cambios en la coherencia a través de los días, la coherencia fue mayor desde el día 1, y un notable mayor acoplamiento funcional de SM con GD y SUM fue observado en el análisis global, lo cual podría indicar que la comunicación del septo medial con estas dos regiones es importante para el procesamiento de información espacial.

En estudios previos se ha mostrado que la serotonina participa en la modulación de la actividad theta hipocampal en relación con el procesamiento de información (Olvera-Cortés et al., 2013). En particular, la reducción serotoninérgica en el hipocampo facilito el aprendizaje en el LAM, asociado con mayor potencia de la actividad theta de alta frecuencia (6.5-9.5 Hz) en CA1 (Gutiérrez-Guzmán et al., 2011). Un efecto similar de la PR fue encontrado en la memoria de trabajo espacial después de reducir el contenido de serotonina septal (López-Vázquez et al., 2012). El presente y previos estudios apoyan la hipótesis del papel del sistema serotoninérgico como desincronizador de la actividad theta hipocampal, dentro del cual un bajo tono serotoninérgico podría conducir actividad theta y la codificación de información (Kocsis y Vertes, 1997; Vertes, 2005). Sin embargo, la reducción de serotonina del SUM/HP (Gutiérrez-Guzmán et al., 2012), o solo en el SUM

(Hernández-Pérez et al., 2015), produce deficiencias en el aprendizaje y la ausencia cambios en la actividad theta en CA1 relacionados al aprendizaje y reducida comunicación entre el SM y CA1, deteriorando la consolidación de la memoria. Lo anterior podría indicar que la serotonina actúa en el modelo de la desincronización, pero solo en relevos del sistema donde la frecuencia theta ya ha sido codificada.
Terminales serotoninérgicas de neuronas del rafé, hacen sinapsis sobre neuronas septo hipocampales en las cuales su actividad puede ser modulada por receptores serotoninérgicos (Milner y Veznedaroglu, 1993). Ha sido mostrado que serotonina liberada en SM normalmente inhibe el disparo rítmico de células septales, produciendo desincronización hipocampal (Kinney et al., 1996). También se ha demostrado la participación de diferentes receptores serotoninérgicos en la modulación de la actividad theta, principalmente por la aplicación de antagonistas al receptor 5HT1A, 5HT2A y 5HT2C que producen un ritmo theta hipocampal y aumento de las oscilaciones theta en SM, mientras que en contraste, la administración de agonistas inhiben el disparo de neuronas registradas en SM y suprimen el ritmo theta hipocampal y del SM (Kazmierska y Konopacki, 2015; Kehne et al., 1996; Hajos et al., 2003; Sörman et al., 2011). Es probable que la reducción de serotonina septal desinhibiera neuronas del SM y así facilitara la ocurrencia de la actividad theta asociada con el aprendizaje, lo cual podría indicar una regulación tónica del sistema septo-hipocampal por serotonina.

Por otro lado, la potencia relativa fue determinada en la condición basal (atento-quieto) registrada en la caja de mantenimiento durante los diferentes días, antes de iniciar el entrenamiento. Primero es notable que la PR del SM, GD, CA1, SUM y MM en la condición basal no cambió durante los días de entrenamiento en los grupos vehículo y 5HT-D; sin embargo, en el análisis entre grupos se mostró que el grupo 5HT-D presentó menor PR en las frecuencias 6.5 y 7 Hz solo en el GD del hipocampo cada día. En relación con estos datos, dos tipos de actividad theta hipocampal han sido distinguidos por su farmacología y conducta. La actividad theta tipo 1 (resistente-atropina) está asociada con el movimiento voluntario y conducta exploratoria (caminar, correr, exploración visual y cambio postural), mientras que la

actividad theta tipo 2 (sensible-atropina) se presenta durante inmovilidad (conducta reflexiva, acicalar y comer) y durante anestesia con uretano (Vanderwolf, 1969; Kramis et al., 1975). Se sugiere que a pesar de que la actividad theta tipo 2 es dependiente del sistema colinérgico, la reducción de la PR de la actividad theta en el grupo 5HT-D podría tener un componente serotoninérgico, el cual podría estar asociado con el estado preparatorio del movimiento (Vanderwolf, 1969). Recientemente, se ha mostrado que la activación in vitro del SM genera un ritmo theta hipocampal sensible-atropina y resistente-atropina (Goutagny et al., 2008) y la participación de células septales GABAérgicas es importante para la conducta exploratoria y para la actividad theta tipo 2 (Gangadharan et al., 2016).

Por otro lado, se ha reportado que la actividad theta está asociada con el movimiento y velocidad de desplazamiento, y puede cambiar su potencia y frecuencia (McFarland et al., 1975; Muir y Bilkey, 2003; Shin et al., 2001) de manera que entre más rápido corre un animal, la frecuencia theta es mayor; sin embargo, la actividad motora desplegada por los animales de los dos grupos fue similar y no se observaron diferencias significativas en la velocidad durante los días de entrenamiento (Figura 13D) y la correlación de la velocidad con frecuencia pico theta del GD y CA1 no fue significativa en ninguno de los dos grupos (Figura 18B). Esto indica que los cambios observados en la frecuencia theta posiblemente están más asociados con el proceso cognitivo sin descartar el procesamiento de información sensorial-motora que también ha sido integrada (Andersen, 2007; Bland y Oddie, 2001; Richard et al., 2013). En apoyo a esto, se ha mostrado que ocurren cambios en la frecuencia theta durante la ejecución de una tarea de aprendizaje de lugar, pero no en el aprendizaje de señal (Olvera-Cortés et al., 2002, 2004), y ocurre un incremento en las oscilaciones theta durante el proceso cognitivo de la toma de decisiones (en tarea espacial) descartando la velocidad (Belchior et al., 2014).

9. CONCLUSIÓN

Finalmente, en el presente estudio se demuestra que la reducción de serotonina septal facilita la codificación de información durante los primeros días del entrenamiento en asociación con cambios en la frecuencia theta y un mayor acoplamiento funcional de le septo medial con el hipocampo y núcleos mamilares.

La modificación de la serotonina del septo medial repercute de manera muy importante en la función del septo medial, de manera que puede facilitar o afectar la comunicación del septo con el hipocampo y núcleos mamilares para hacer más eficiente o afectar la codificación de información.

Este estudio resalta la importancia que tiene la serotonina en la modulación de la actividad theta hipocampal y el aprendizaje dependiente del hipocampo.

10. REFERENCIAS

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Research report

Serotonergic modulation of septo-hippocampal and septo-mammillary theta activity during spatial learning, in the rat

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HIGHLIGHTS

- Septal serotonin depletion (5HT-D) facilitates the acquisition of spatial learning.
- 5HT-D increases the hippocampal theta frequency during the spatial learning.
- 5HT-D results in higher septo-hippocampal theta coherence during the learning.
- 5HT-D results in higher septo-mammillary theta coherence during the learning.
- Serotonin, acting on the medial septum, modulates hippocampal theta activity and spatial learning.

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ABSTRACT

Theta activity has been related to the processing of spatial information and the formation of hippocampus-dependent memory. The medial septum (MS) plays an important role in the control and coordination of theta activity, as well as in the modulation of learning. It has been established that increased serotonergic activity may desynchronize theta activity, while reduced serotonergic activity produces continuous and persistent theta activity in the hippocampus. We investigate whether serotonin acting on the medial septum could modify spatial learning and the functional relationship between septo-hippocampal and septo-mammillary theta activity. The serotonin was depleted (5HT-D) from the medial septum by the injection of 5,7 DHT (5,7- dihydroxytryptamine). Theta activity was recorded in the dorsal hippocampus, MS and mammillary nuclei (SUM, MM) of Sprague-Dawley male rats during spatial learning in the Morris water maze. Spatial learning was facilitated, and the frequency of the hippocampal theta activity during the first days of training increased (to 8.5 Hz) in the 5HT-D group, unlike the vehicle group. Additionally, the coherence between the MS-hippocampus and the MS-mammillary nuclei was higher during the second day of the test compared to the vehicle group. We demonstrated that septal serotonin depletion facilitates the acquisition of spatial information in association with a higher functional coupling of the medial septum with the hippocampus and mammillary nuclei. Serotonin, acting in the medial septum, modulates hippocampal theta activity and spatial learning.

1. Introduction

Hippocampal theta activity is a regular electroencephalographic oscillation from 4 to 12 Hz that can also be recorded in other mesencephalic and diencepahlic structures that are part of the ascending synchronising system [19,112,101,117]. Hippocampal theta activity has been associated with a functional role in the representation and processing of spatial information in memory and other cognitive processes [44,21,8,54,90]. In particular, changes in efficiency in place learning tasks had been related to changes in the power and frequency of hippocampal theta activity [96,91,93,20,104,124]. As part of the ascending synchronising system, the medial septum and diagonal band of Broca (hereafter known as the medial septum, MS) is a critical information relay station in the circuit

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connecting the hippocampus and other subcortical limbic regions, such as the median mammillary and supramammillary nuclei (MM and SUM, respectively) [50,97]. SUM has been implicated in hippocampal theta frequency encoding [59,81,62] and spatial learning [105,96,107,40] while the MM has neurons that fire rhythmically in phase with the hippocampal theta, which are driven by descending projections from the hippocampus [10,64]. The MS is considered the pacemaker of the theta rhythm, as it has a determining role in the temporal coordination of the hippocampus information flow and in the generation of the theta rhythm [36,4,58,123]. The inhibition of the MS cell activity with lidocaine abolishes theta oscillations in the hippocampus and entorhinal cortex [4,74,68] and results in severe deficits in spatial learning [75,124]. Thus, the MS regulates spatial hippocampal representation [77]. Selective medial septal lesions on GABAergic and/or cholinergic cells impair spatial learning in the Morris water-maze [18,29,98], reduce the power of hippocampal theta activity and result in the loss of rhythmic firing of septo-hippocampal neurons [69,6,43,110].

In parallel with the ascending synchronising system, there is a serotonergic system that originates from the dorsal and medial raphe nuclei; the median raphe nucleus (MR) has extensive projections to the MS and hippocampus that express different types of receptors to serotonin [72,83,115,79]. This system has been proposed to be directly involved in hippocampal EEG desynchronization or the non-theta state [122,117,71,121]. Based on experiments in anesthetized and freely moving rats, electrical stimulation of the MR has been shown to inhibit the firing of hippocampal pyramidal cells [106], to disrupt the bursting discharge of septal pacemaker cells [7,119], to decrease theta expression in the medial septal area and to desynchronize the hippocampal EEG [60,119]. In addition, the selective systemic administration of 5HT2C receptor agonist inhibits theta oscillations of the MS and theta activity in the hippocampus [42]. In contrast, continuous and persistent hippocampal theta activity has been shown after the inhibition, temporal inactivation (with lidocaine or procaine) or selective application of 5HT1A serotonin receptor agonist to the MR in anesthetized rats and awake rabbits [114,60,116].

In relation to the functional role of serotonin as a desynchronizer of hippocampal theta activity, it has been proposed that information arriving in the absence of theta (desynchronization) is not encoded [117]; therefore, serotonin could block or temporally suspend memory processes in the hippocampus and, to a certain extent, is responsible for the ability to ignore non-significant environmental events [120]. In addition, it was proposed that a high serotonergic tone in the medial septum could prevent coding information, while a low tone could facilitate the encoding and consolidation of information [53]. However, these hypotheses have not been simultaneously tested in experiments during information processing related to spatial learning and memory, with records of the underlying theta activity.

The participation of serotonin in the modulation of hippocampal theta activity in relation to information processing has been demonstrated in the hippocampus and supramammillary nucleus [94,39,40,47]. However, because of the important role of the MS on the theta activity and spatial learning and the participation of serotonin as possible desynchronizer of hippocampal theta activity, it is important to know the functional role of serotonin on the medial septum and its impact on theta activity of the septo-hippocampal and septo-mammillary circuit, especially during behaviour involving the encoding and retrieval of information, such as learning and memory. We selectively depleted serotonin in the medial septum, and the EEG was simultaneously recorded in the hippocampus, medial septum and mammillary nuclei during place learning in the rat.

2. Materials and methods

2.1. Animals

A total of 29 male Sprague Dawley rats weighing between 400 and 460 g were used in this study. The rats were maintained under a normal light/dark cycle (12 h/12 h) at a temperature of 22 °C with free access to water and food. In the room colony, four rats were housed per cage, after the surgery one rat was housed per cage until the end of the experiments. Eight rats were assigned to the experimental group in which the serotonin was depleted (5HT-D) from the medial septum by the injection of 5,7-DHT and eight rats were assigned to the vehicle group. The remaining rats did not meet the inclusion criteria (serotonin reduction greater than 50% compared to the vehicle group, the proper position of the electrodes in each recording site) and were discarded. The order in which the rats started the behavioural test was counterbalanced. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and for the "Norma Oficial Mexicana" for the use of experimental animals (NOM-062-ZOO-1999). All the experiments were approved by the Research Ethics Committee of the Instituto Mexicano del Seguro Social.

2.2. Surgery

The rats were deeply anesthetized with Ketamine (60 mg/kg i.m.) and sodium pentobarbital (14 mg/kg i.p.) before stereotaxic surgery. The rats were depleted of serotonin through the micro-infusion of 5,7-dihydroxytriptamine (5,7-DHT) into the MS according to the Atlas of [99], (coordinates: same as the recording electrode) (1.5 μg dissolved in 0.1 μl of 0.1% ascorbic acid in a saline solution) at an infusion rate of 0.1 μl/min for 5 min using a Hamilton 10-μl syringe located on an infusion pump. Thirty minutes before the infusion of 5,7-DHT, the rats received pargyline (30 mg/kg, i.p.) in order to protect the noradrenergic terminals [14,15,95,28]. The other rats received the vehicle solution infusion (vehicle group), in a similar volume and rate as the 5HT-D group. In the same surgery, the rats were chronically implanted with bipolar concentric electrodes in the MS (coordinates: 0.6 mm anterior from the bregma,1.5 mm right to the midline,15° from vertical and 6.8 mm dorsoventral from the cranial surface or DVC); the dentate gyrus (DG, coordinates: 3.5 mm posterior to the bregma or PB, 1.5 mm lateral from the midline and 3.6 mm DVC), CA1 (coordinates: 4.5 mm PB, 2.4 mm lateral from the midline and 2.6 mm DVC) from the hippocampus; the SUM (coordinates: 4.6 mm PB, 0-0.2 mm lateral from the midline and 8.24 DVC) and MM (coordinates: 4.6 mm PB, 1.3 mm lateral left from the midline, 13° from vertical and 9.2 DVC). The electrodes were made of nichrome wire (60 μm) located in a stainless steel #30 calibre cannula isolated with epoxy resin with a small surface exposed on the tip. The electrodes were fixed to the skull with dental acrylic, and one screw was placed in the frontal bone for grounding. After the surgery, a combination of antibiotics, analgesics, antipyretics and expectorants was administered (Respivet Senosiain 0.1 ml/kg i.m). After a 15-day recovery period, the behavioural test began.

2.3. Behavioural test

To evaluate spatial learning, the Morris water maze was used, which consisted of a circular pool (150 cm in diameter) filled with water maintained at 27 +–1 °C and dyed dark blue by the addition of gentian violet. The pool contained a circular platform that was 10 cm in diameter, and the surface was placed 2 cm under the water level in a fixed position in one quadrant. Stimuli were located around the maze in the room.

The test consisted of four trials per day with an inter-trial interval of two minutes for 6 consecutive days. Each trial began with placing the rat into the pool in one of the quadrants facing the wall (the starting quadrants were randomly chosen each day but were similar for all rats within one day). If, during the course of each trial, the rat did not find the platform within 60 s, it was placed on the platform where it remained for a period of 15 s. Each inter-trial interval, the rats were dried with a towel. Twenty-four hours after the last test day, a transfer test or probe trial was performed, which involves placing the animal in the pool without the platform for a period of 30 s. Swimming routes were recorded with a video camera and stored in a computer with the software video-bench (DataWave Technologies). The escape latencies, distance travelled, and swimming velocity, were obtained from each rat. The mean distance swum, swimming velocity, and the mean daily latencies from the four daily trials were compared. In the probe trial, the number of crosses made by the rat into the central annulus of each quadrant (corresponding to the platform's position and area) was counted and compared.

2.4. Recording sessions

The rats were connected to a commutator (Neuro-Tek, CA. IT) using a cable with a male connector. The commutator was connected to one amplifier (Neurodata acquisition system, GRASS Mod 15A54, Astro Med Inc. 600 E. Greenwich Ave., W. Warwick, RI 02893, USA). The EEG signals were digitalized with a sampling frequency of 1024 Hz (DataWave Technologies data acquisition system) and the band-pass filter was set to 1–100 Hz. A notch filter at 60 Hz was applied to eliminate the line noise. The signal was stored in a computer, to be analysed offline. The data were imported into MATLAB environment (Mathworks, Inc.). Segments with artefacts (principally caused by the head shaking of the rats) were visually identified and eliminated using the EEGLAB software [32].

Before the first trial of each training day, an EEG record was made in a baseline condition (awake-immobile) with the wet rat in a cage for a period of 60 s. After that, an EEG during the platform search was recorded during each trial. The signal of each recording region was analysed by the Fast Fourier Transform. Absolute power was obtained as the mean spectrum of two-second samples, to ensure a resolution of 0.5 Hz. The relative power (RP) was obtained for each 0.5 Hz of frequency as the percent of the total 4–12 Hz power band. The average RP at each frequency of the four daily trials of each test day was determined.

Comparisons were made of the RP in the range of 4 to 12 Hz obtained in each region by day, frequency and behavioural state in each group (intra-group comparisons) using ANOVA for repeated measures and *t*-tests, with Bonferroniís correction for paired tests; the inter-group comparisons of the RP were made using an ANOVA for repeated measures considering the factors group and frequency as independent and the training days (1–6) as repetitions. The comparisons of coherence between the signals from different recording regions were performed in a similar manner to that of the RP values. The coherence was determined between the medial septum and hippocampus (MS-DG, MS-CA1) and the medial septum and mammillary nuclei (MS-SUM, MS-MM) across the different days of training. Both the power and the coherence values of the EEG were obtained using custom programs adapted from Ken's MATLAB library written by Ken Harris, available at: http://osiris.rutgers.edu/Buzsaki/software

2.5. Neurochemical-serotonin concentration

High-performance liquid chromatography (HPLC) was used to verify the concentration of serotonin and metabolite 5-hydroxy indole acetic acid (5HIAA) in medial septum tissue. After completing the behavioural test, the rats were sacrificed, a brain slice was removed and punching was performed selectively for the extraction of the medial septum tissue with a micropipette tip. The tissue samples were homogenised in HCl 1 N and were centrifuged. Then, the supernatant was removed and was filtered. The concentrations of serotonin and 5HIAA (pg/mg) were determined using a Lichrocart purospher star column (150 ˗ 4.6, RP – 18 end caped, 5 mm, MERK KGa A, Darmstadt; Germany) and an electrochemical detector (AtecLydenVT-03) with a work potential of 0.800 mV adjusted to the pH of the mobile phase. The mobile phase was composed of citric acid (50 mM), H3PO4 (50 mM), EDTA (20 mg), octanesulfonic acid (120 mg/L) and methanol (10%) at pH 3.1.The flow rate was 1.5 ml/min. The concentrations are expressed in pg/mg.

During the process of septal tissue extraction for HPLC, the position of the electrode on the MS was visually verified while the rest of the brain tissue was placed in a fixative solution (10% formaldehyde in phosphate buffer) for several days to histologically verify the proper position of the electrodes in each recording site, and 30-μm slices were made in a microtome (*Microm HM325*) and were stained with cresyl violet.

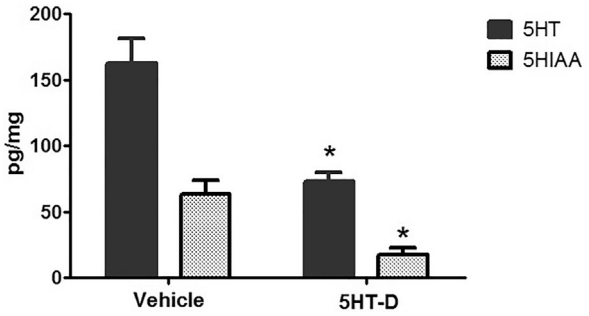

Fig. 1. The concentration of septal 5-HT and 5-HIAA (pg/mg of fresh tissue). Mean ± S.E.M. *, 5HT-D vs. vehicle.

3. Results

3.1. Verification of the serotonin concentration and the position of the electrodes

Fig. 1 shows the concentrations of serotonin and 5HIAA in the medial septum in the two groups. The 5HT-D group had significantly lower concentrations compared with the vehicle group ($t = 4.190$, $df = 7$, $p = 0.0041$) for serotonin and ($t = 4.069$, $df = 7$, $p = 0.0048$) for 5HIAA. The position of the electrodes in each region was checked and two vehicle and two 5HT-D rats were discarded. Fig. 3A represents the electrode positions in the hippocampus and mammillary nuclei for different rats.

3.2. Behaviour

Escape latency on the training day was compared in each group using a Friedman's

ANOVA and Wilcoxon's test. Fig. 2A shows that both groups had significantly reduced escape latency through days two to six with respect day 1: the vehicle group ($\chi^2_r = 28.357$ $P = 0.0001$) on day 2 ($p = 0.025$) and days 3–6 ($p = 0.012$); and the 5HT-D group ($\chi^2_r = 28.857$ $P = 0.001$) on days 2–6 (0.012). The inter-group analysis using the Mann Whitney *U* test showed a significant difference between groups ($\sum R_x = 1245$ $P = 0.017$), on day 2 ($p = 0.001$), day 3 ($p = 0.036$), and an interesting tendency on day 1 ($p = 0.058$).

The distance travelled was analysed by training day using ANOVA for repeated measures and the t-Test with Bonferroni adjustment as a post hoc test. Fig. 2B shows the intra-group comparisons of the distance travelled. The vehicle group ($F_{5,35} = 25.45$,

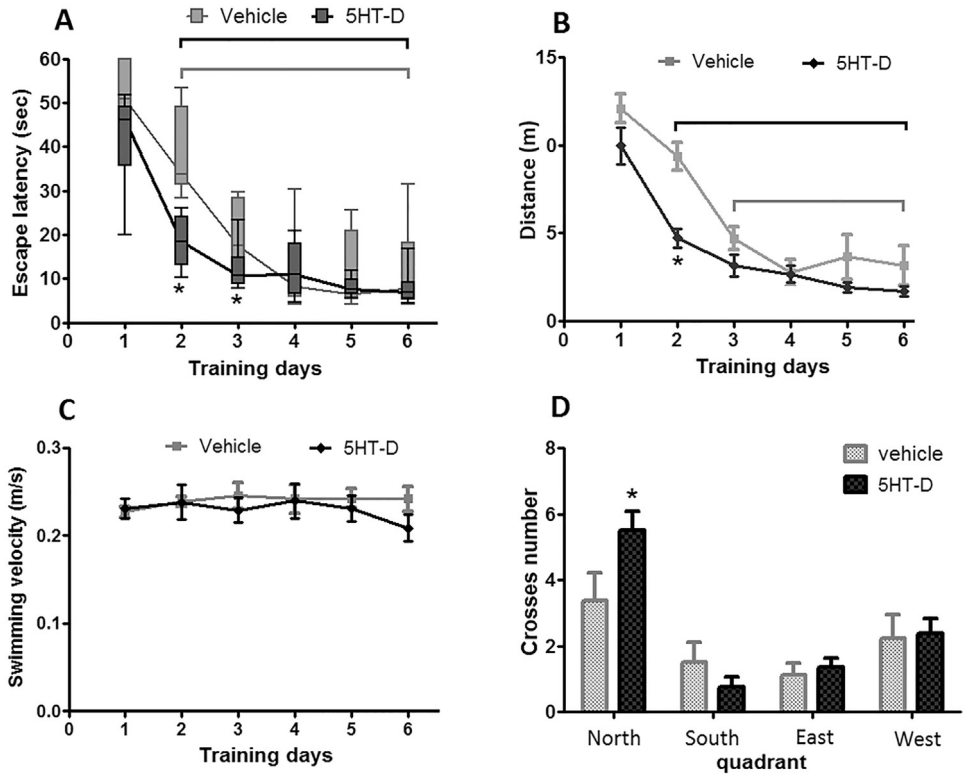

Fig. 2. A comparison of learning performance among the two groups of rats. (A) Escape latency each training day. Median ± S.E.M. (B) Distance travelled by each group during the training days. Mean ± S.E.M. *, 5HT-D vs. the vehicle. ⌐ Day 1 vs. the subsequent days in the vehicle group. ¬ Day 1 vs. the subsequent days in the 5HT-D group. (C) Swimming velocities displayed by the two groups of rats during the training days. Mean ± S.E.M. (D) Transference test, and the number of crosses into the central annulus in each quadrant (corresponding to the platform's position). Mean ± S.E.M. *, 5HT-D vs. the vehicle.

$P < 0.0001$) reduced their distances travelled from day 3 ($p < 0.01$) to 6 ($p < 0.001$, days 4 to 6) with respect to day 1. However, remarkably, the 5HT-D group significantly reduced their distances travelled ($F_{5,35} = 38.59$, $P < 0.0001$) a day earlier, on days 2–6 ($p < 0.05$ all days) and began with shorter distances from the first day. A two way ANOVA for repeated measures (with factors group and day) was used for the inter-group analysis and a significant interaction between factors was observed ($F_{5,70} = 2.708$, $P = 0.0270$). The 5HT-D group travelled a significantly shorter distance on day 2 ($p < 0.001$) with respect to the vehicle group.

A similar comparison to those of the distance travelled was conducted for swimming velocity and no changes were observed in any group by training day, as shown in Fig. 2C ($F_{5,35} = 0.3646$, $P = 0.8693$), for the vehicle group; or for ($F_{5,35} = 0.5348$, $P = 0.7485$) the 5HT-D group. The intergroup comparison did not show significant differences between groups ($F_{5,70} = 0.5403$, $P = 0.7451$).

To compare the number of crosses into the central annulus in each quadrant (corresponding to the platform's position) in the transfer test on day 7, a two way ANOVA (group −quadrant) and t-Test with Bonferroni adjustment post-tests were used. We found a tendency in the interaction ($F_{3,42} = 2.698$, $P = 0.057$), and as shown in Fig. 2D, the 5HT-D group had a significantly greater number of crosses in the north quadrant where the platform was located ($p < 0.05$) with respect to the vehicle group.

3.3. EEG theta power

The EEG results recorded in different regions during baseline condition (in a holding cage awake-immobile) and during the searches for the platform in the water maze each day of training were analysed.

Fig. 3B shows a representative trace of an EEG from the different recording sites and a spectrogram of the CA1 region (Fig. 3C), of one vehicle group rat and one 5HT-D group rat, during the baseline condition and while searching for the platform (swimming) on day 2 of training. The predominance of theta activity (peak power approximately 7.5 Hz) during the search for the platform in the Morris water maze can be observed in both groups, whereas irregular activity prevailed during the baseline condition.

3.3.1. Dentate gyrus and CA1

Intra-group comparison of RP of each recorded region wasmade using an ANOVA for repeated measures (with factors frequency and day) and t-Test with Bonferroni adjustments as the post-test. Fig. 4 shows the intra-group comparison of RP for the vehicle group: similar changes in the RP of the DG and CA1 regions were observed across the days of training in the water maze (DG, $F_{80,510} = 4.163$, $P < 0.0001$; CA1, $F_{80,510} = 6.685$, $P < 0.0001$). Significant increases in the power at high frequencies were observed from day 3 to 6 compared to day 1 (approximately 7.5-8-8.5 Hz), while low frequencies (5.5-6.5 Hz) decreased with respect to day 1. Thus, the RP of the theta activity changes at specific frequencies during the training days.

The intra-group comparisons of the RP (ANOVA for repeated measures, with factors frequency and day, and t-Test with Bonferroni adjustment as post-tests) in the 5HT-D group showed similar changes in DG and CA1 during the days of training (DG, $F_{80,595} = 6.069$, $P < 0.0001$; CA1, $F_{80,595} = 4.765$, $P < 0.0001$); however, interestingly the RP of the high frequency theta activity (7.5 and 8 Hz) increased since day 2, and the low frequencies (6-6.5 Hz) were reduced with respect to day 1. That is, the changes observed in the vehicle group on day 3 were observed earlier (on day 2) in the 5HT-D group. On days 3 to 6, the power and frequencies con-

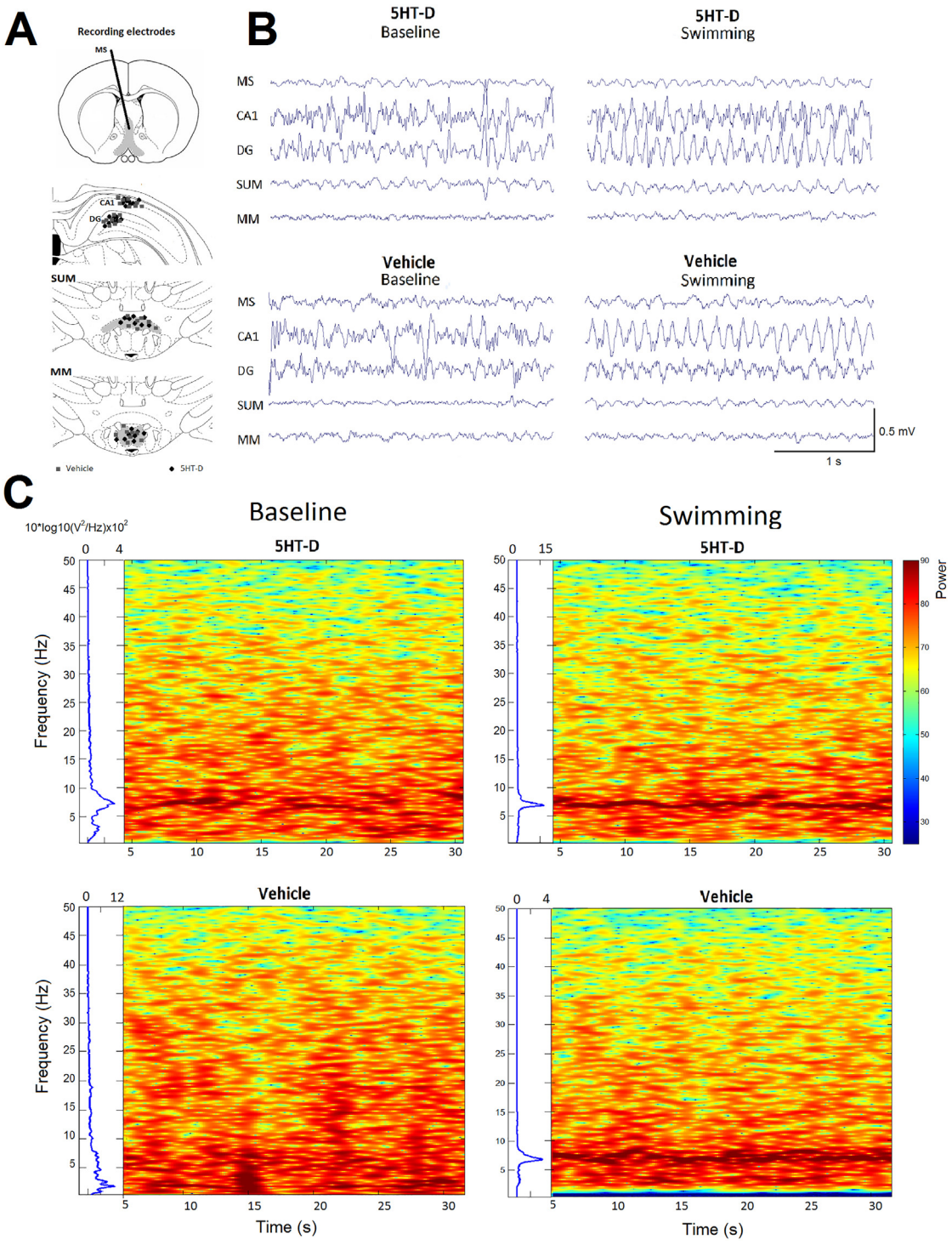

Fig. 3. (A) Electrode positions from each recorded site for different rats separately in each group and (B) EEG traces of one representative rat from each group. Three-second samples recorded during baseline conditions (immobile-awake in a holding cage) and during the search (swimming) for the platform on day 2. Cal. 0.5 mV/1 s. MS, medial septum; DG, Dentate gyrus; CA1, Corn Ammon; SUM, supramammillary nucleus; MM, mammillary nucleus. (C) The time-frequency spectrogram of a representative trace of EEG of one representative rat from each group on day 2 in the two behavioural conditions.

tinued to increase (from 8 to 8.5 to 9 Hz) while the low frequencies (6–7 Hz) significantly decreased ($p < 0.001$) with respect to day 1.

In general, both groups had low frequencies on day 1, and as their training elapsed, an increase in the power and frequencies was observed, which was associated with the efficiency observed in the behaviour, particularly with the distance travelled. Values of significance for differences between training days in the different regions recorded are shown in Table 1. In Fig. 4, the RP of the DG and CA1 in the first 3 days (principal differences in behaviour) and on day 6 (when learning was established; the results observed on days 4 and 5 were similar to those from day 6) are presented. A two way ANOVA (group – frequency) and t-Test with Bonferroni adjustment as post-tests were used to the global analysis of the RP of all 6 days of training (the average relative power in each theta frequency for every day) of the two groups, and it was observed that the 5HT group theta activity had a higher frequency (8.5 Hz) in

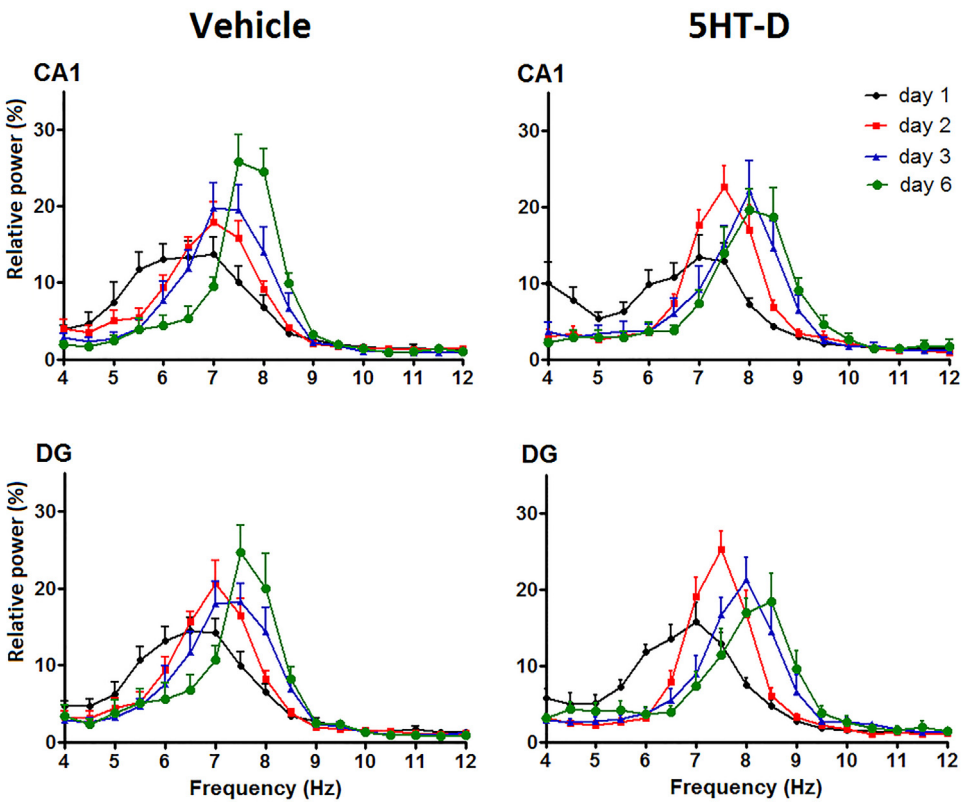

Fig. 4. The relative power of theta activity (4–12 Hz) recorded in the DG and CA1in the vehicle and 5HT-D groups each training day during the search for the platform. Only days 1, 2, 3 and 6 are shown. Mean ± S.E.M. Significant differences are listed in Table 1 and include the other recorded site. $p < 0.05$.

Table 1
Significant changes of the RP of each group (intra-group comparisons).

Region/day		D1vsD2	D1vsD3	D1vsD4	D1vsD5	D1vsD6
MS	Vehicle	ns	ns	ns	ns	8
	5HT-D	/7.5–8	ns	ns	ns	ns
DG	Vehicle	ns	/7.5–8	5.5–6.5/7.5-8.5	5.5–6.5/7.5-8	5.5–6.5/7.5-8
	5HT-D	6–6.5/7.5-8	6–7/8-8.5	6–7/8-9	6–7/8-8.5	6–7/8-9
CA1	Vehicle	5.5/	5.5/7–8	5.5–6.5/7.5-8.5	5.5–6.5/7.5-8	5.5–6.5/7.5-8.5
	5HT-D	6/7.5–8	6/8–8.5	6–7/8-8.5	6–7/8-8.5	6–7/8-9
SUM	Vehicle	ns	ns	5.5–6.5/7.5-8.5	5.5–6.5/7.5-8	5.5–6/7.5-8.5
	5HT-D	/7.5–8	/8–8.5	/8–8.5	/8–8.5	8–9
MM	Vehicle		5.5/7.5	5.5–6/7.5-8	5.5–6/7.5-8	5/7.5–8
	5HT-D	/7.5–8	/8	/8	/8–8.5	/8–8.5

Comparisons between the day 1 and all other days of training (D1vs D2 to D6). Values before the diagonal are significantly reduced, after the diagonal are significantly increased. Frequency values in Hz. ns, no significant. $p < 0.05$.

the DG, CA1 and SUM with respect to the vehicle group during the water maze test (DG, $F_{16,85} = 3.890$, $P < 0.0001$; CA1, $F_{16,85} = 4.947$, $P < 0.0001$; SUM, $F_{16,85} = 5.176$, $P < 0.0001$). (Fig. 5, before the black arrow).

Fig. 6A shows the Pearson's correlation between theta frequency peak and travelled distance each training day for each rat. The vehicle group showed a negative correlation between distance and theta frequency peak of the DG ($r = -0.3999$, $P = 0.0087$) and CA1 ($r = -0.5771$, $P = 0.0001$) regions. Similar changes in the correlation between theta frequency peak and distance of the 5HT-D group were observed for DG ($r = -0.5803$, $P = 0.0001$) and CA1 ($r = -0.5679$, $P = 0.0001$). However, in the correlation between theta frequency peak and swimming velocity (6B), the vehicle and the 5HT-D groups did not show a significant correlation throughout the training days in the DG (vehicle: $r = 0.0427$, $P = 0.7880$ and 5HT-D: $r = 0.0339$, $P = 0.8186$) and CA1 regions (vehicle: $r = 0.2652$, $P = 0.0897$; 5HT-D: $r = 0.0230$, $P = 0.8766$).

3.3.2. *Medial septum*

The intra-group analysis (two way ANOVA for repeated measures, with factors frequency and day and t-Test with Bonferroni adjustment as post-tests) of the vehicle group RP showed no significance in the interaction of frequency across days ($F_{80,510} = 1.232$, $P < 0.0972$). In the 5HT-D group, a significant interaction between frequency and days was observed ($F_{80,595} = 2.153$, $P < 0.0001$) and an increase in RP was observed for high frequencies (7.5–8 Hz) only during day 2 (Table 1). It is important to note that on day 2, the travelled distance was significant lower with respect to the distance travelled on day 1 in this group, whereas in the vehicle group, the distance was not reduced until day 3, and increases in the MS RP were not observed in the vehicle group.

3.3.3. *The supramamillary and medial mammillary nuclei (SUM-MM)*

The intra-group analysis (two way ANOVA for repeated measures, with factors frequency and day, and t-Test with Bonferroni

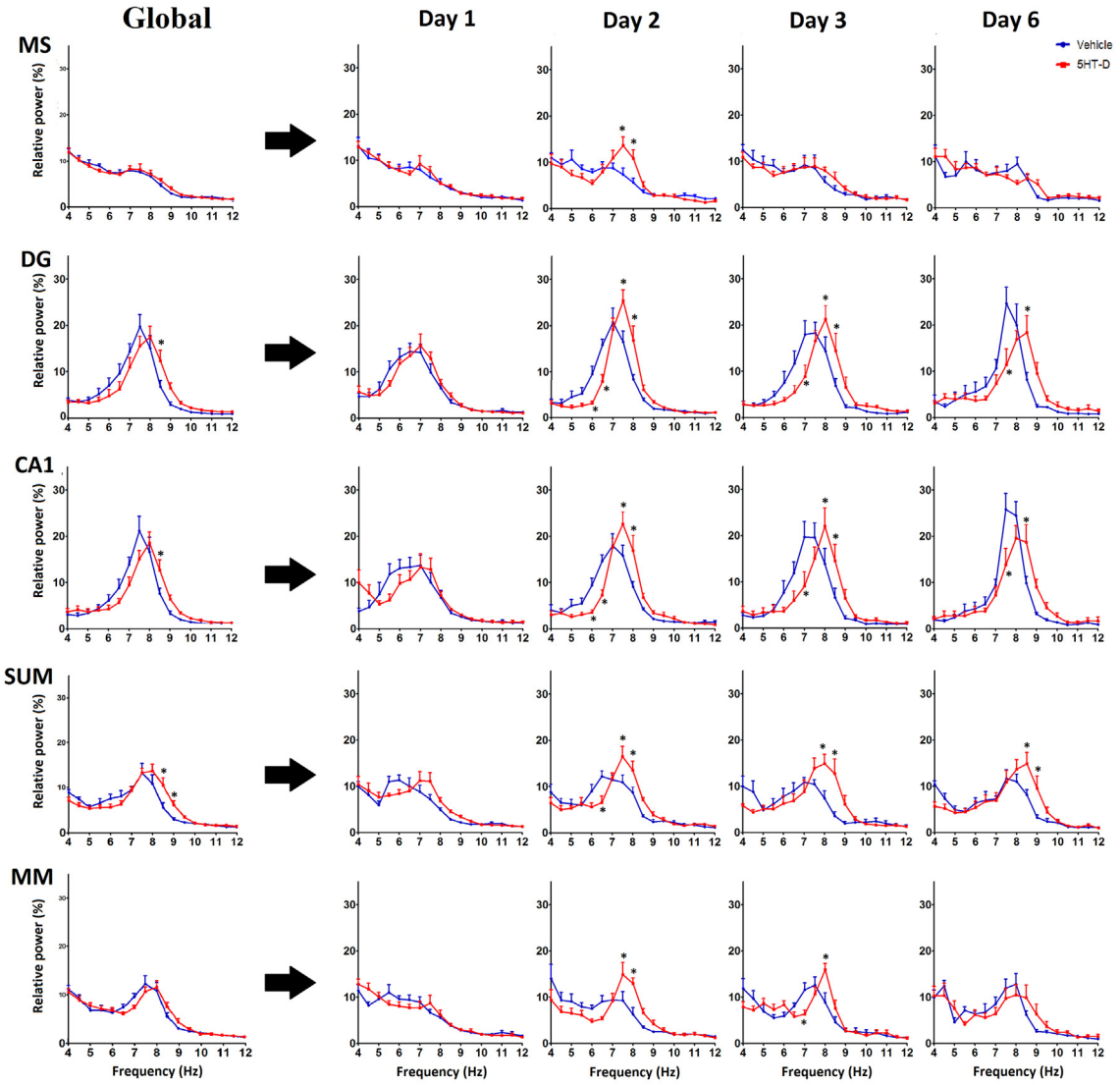

Fig. 5. Left: The relative power and frequency of the MS, DG, CA1, SUM and MM EEG during the global performance (main effect) on all days in each group. Right: After the arrow, the relative power of the theta activity (4–12 Hz) recorded in the same regions in each group during the search for the platform during the training days. Only days 1, 2, 3 and 6 are shown. Mean ± S.E.M. *, 5HT-D vs the vehicle. $p < 0.05$.

adjustment as post-tests) of the vehicle group showed significant changes in RP when compared to the frequency on the training days (SUM, $F_{80,510} = 5.706$, $P < 0.0001$; MM, $F_{80,510} = 3.588$, $P < 0.0001$). Particularly on days 4, 5 and 6, the high frequency RP increased (7.5-8.5 Hz) and the low frequency RP decreased (5.5-6.5 Hz) with respect to day 1. The 5HT-D group experienced changes in frequency over the training days (SUM, $F_{80,595} = 3.018$, $P < 0.0001$; MM, $F_{80,595} = 2.967$, $P < 0.0001$), but in contrast to the vehicle group, the high frequency RP (7.5–8 Hz) significantly increased since day 2 with respect to day 1, and the high frequency (8-8.5–9 Hz) RP increased with respect to day 1 on all other training days, as shown in Table 1. It is notable that the frequency of SUM RP increased during the first days (2 and 3) in the 5HT-D group, which is different from the vehicle group.

Together with the changes observed in the DG and CA1 RP, the possible significance of this result is that the reduction of serotonin in the medial septum facilitates an increase in the high frequency RP in different regions, primarily on day 2. These differences are shown in Fig. 5 (after the black arrow). The inter-group analysis (ANOVA for repeated measures considering the factor group and frequency as independent and the training days as repetitions) showed a significant effect on the interaction of the factors, frequency, group and day in the RP of the hippocampus (DG, $F_{80,1105} = 2.599$, $P < 0.0001$; CA1, $F_{80,1105} = 2.861$, $P < 0.0001$) and mammillary nuclei (SUM, $F_{80,1105} = 1.853$, $P < 0.0001$; MM, $F_{80,1105} = 3.102$, $P < 0.0001$). Paired comparisons showed differences at high frequencies (7.5–8 Hz) on days 2, 3 and 6 ($p < 0.001$) in the different regions. It was found a tendency in the interaction of the group, frequency and day in the RP of MS ($F_{80,1105} = 1.253$, $P = 0.061$) differences were evident in the paired comparisons on day 2 at 7.5 Hz and 8 Hz frequencies ($p < 0.01$).

The baseline conditions recorded (awake-immobile) for the wet rat in a cage for a period of 60 s before the Morris water maze test were analysed. The intra-group analysis (two way ANOVA for repeated measures with factors frequency and day and t-Test with Bonferroni adjustment as post-tests) of the RP of MS (Vehicle, $F_{80,544} = 0.3909$, $P = 1.000$; 5HT-D, $F_{80,640} = 0.7963$, $P = 0.8997$), DG (Vehicle, $F_{80,544} = 0.5552$, $P = 0.9993$; 5HT-D, $F_{80,640} = 0.7158$, $P = 0.9688$), CA1 (Vehicle, $F_{80,544} = 0.6167$, $P = 0.9958$; 5HT-D, ($F_{80,640} = 0.4836$, $P = 1.000$), SUM (Vehicle, $F_{80,544} = 0.7117$, $P = 0.9700$; 5HT-D, $F_{80,640} = 0.5313$, $P = 0.9997$) and MM (Vehicle, $F_{80,544} = 0.8077$, $P = 0.8819$; 5HT-D,

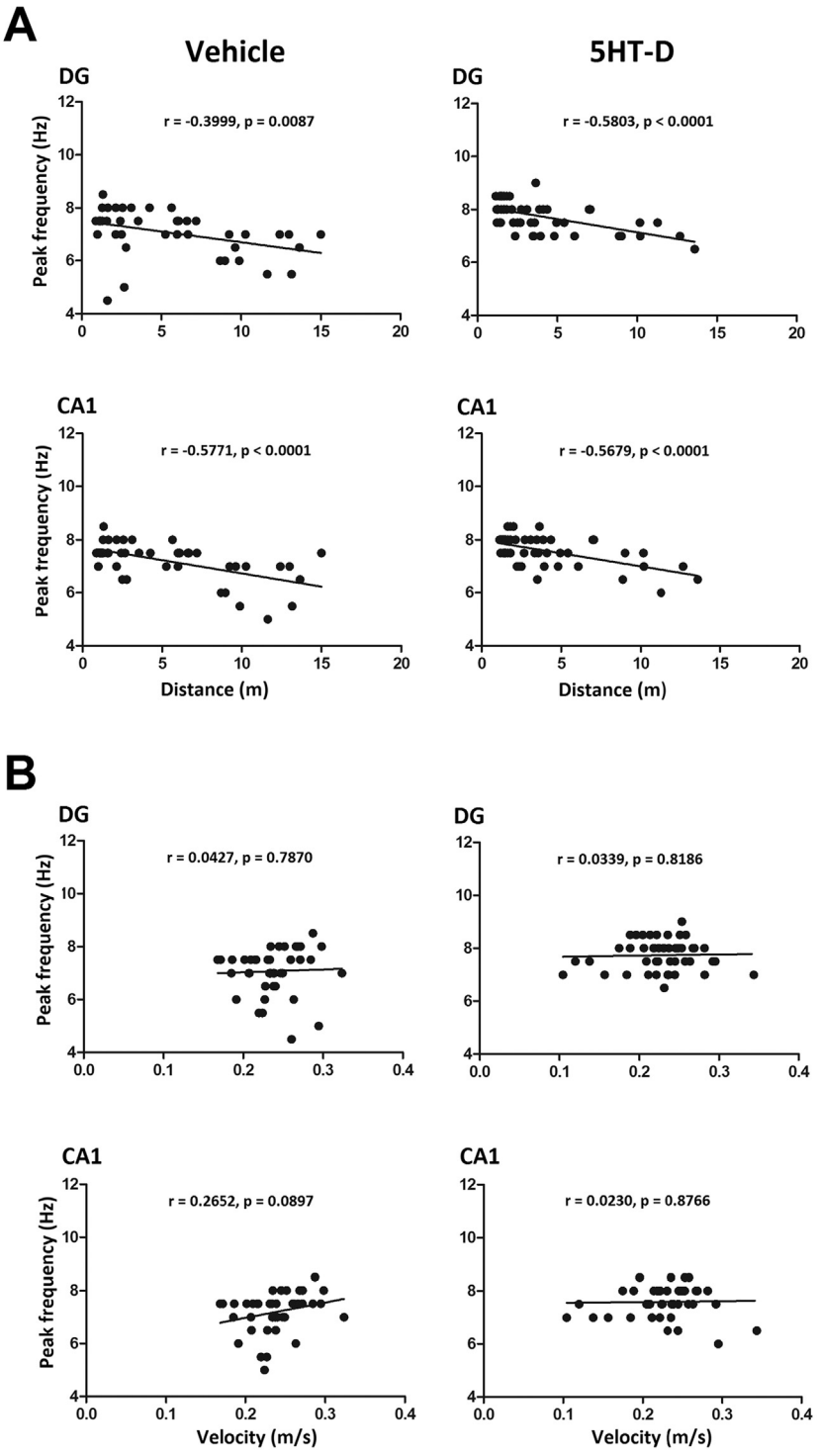

Fig. 6. Pearson's correlation between theta frequency peak and travelled distanceand velocity, in the vehicle and 5HT-D groups of rats. (A) Significant correlations between theta peak frequency and travelled distance were observed in the DG and CA1 of hippocampus in the two groups of rats. (B) Correlation of velocity with the theta frequency peak of DG and CA1 was not significant in either group. Ordinate: peak frequency (Hz), abscissa: distance (meters), velocity (meters/second).

$F_{80,640} = 0.8635$, $P = 0.7922$) did not show differences across the training days in any group (data not showed). The inter-group comparisons (ANOVA for repeated measures considering the factor group and frequency as independent and the training days as repetitions) showed a significant effect in the interaction frequency, group and day only in DG ($F_{(80,1105)} = 2.861$, $P < 0.0001$) of hippocampus: the 5HT-D group had a minor RP in the 6.5 and 7 Hz frequencies on days 1, 2, 3, 5 and 6 ($p < 0.05$) compared with the vehicle group.

3.4. Coherence of theta activity

The coherence of the EEG activity from the medial septum with the EEG of the different regions was compared considering the factors day and frequency, using a two way ANOVA for

repeated measures and t-Test with Bonferroni adjustment as post-tests. The intra-group analysis (frequency – day) of the coherence between the medial septum and dentate gyrus EEG (MS-DG) of the vehicle group showed a significant interaction of frequency and day ($F_{80,576} = 1.354$, $P = 0.0298$). The paired comparisons, showed greater coherence in the frequencies of 9.5-10.5 Hz ($p < 0.05$) only in day 6 with respect to day 1. Coherence between the medial septum and CA1 EEG (MS-CA1) showed a significant interaction of frequency and day ($F_{80,576} = 1.633$, $P = 0.0070$). Paired comparisons showed increased coherence in the frequencies 8.5–9 Hz on day 4 ($p < 0.001$), in the frequencies 7.5–9 Hz ($p < 0.05$) on day 5 and increased coherence in the frequencies 8–10 Hz ($p < 0.05$) on day 6. The coherence between the medial septum and supramammillary nucleus EEG (MS-SUM) did not have significant changes of frequency across the days of training ($F_{80,576} = 1.220$, $p = 0.1824$), but the paired comparisons showed increased coherence in the frequencies of 7.5-9.5 Hz ($p < 0.05$) on days 4, 5 and 6. Finally, the coherence between the medial septum and mammillary medial nucleus EEG (MS-MM) had a significant interaction of frequency and day ($F_{80,576} = 1.344$, $P = 0.0482$); increased coherence in the frequencies 8–9 Hz was observed on days 4, 5 and 6 ($p < 0.05$) with respect to day 1.

The intra-group comparisons (factors frequency and day) of the coherence of each frequency of the EEG between the MS-DG trough training days in the 5HT-D group showed a significant effect ($F_{80,672} = 1.483$, $P = 0.0061$). Paired comparisons indicated significantly increased coherence on day 2 at the 7.5 Hz frequency ($p < 0.05$) and on day 6 at frequencies of 8 and 9 Hz ($p < 0.05$) with respect to day 1. The coherence between MS-CA1 EEG ($F_{80,672} = 1.359$, $P = 0.0262$) was higher on day 2 in the 7.5 Hz frequency ($p < 0.05$) and on day 6 in the 8 and 9.5 Hz frequencies ($p < 0.05$) with respect to day 1. The coherence of the EEG recorded in MS-SUM ($F_{80,672} = 1.320$, $P = 0.0402$) showed a significant effect on training days; however, paired comparisons showed no significant changes. The coherence between MS-MM EEG ($F_{80,672} = 1.474$, $P = 0.0069$) increased in the 8-8.5 Hz frequencies on day 6 ($p < 0.05$) with respect to day 1. These data are summarized in Table 2.

An analysis of the coherence and frequency of all 6 days of training during the platform search was performed (average coherence and frequency theta every day) in each group to identify the global effect. A two way ANOVA (group – frequency) and t-Test with Bonferroni adjustment as post-tests were used. It is interesting to note that the 5HT-D group presented higher coherence on the EEG recorded in MS-DG ($F_{16,85} = 1.305$, $P = 0.0213$) and MS-SUM ($F_{16,85} = 1.504$, $P = 0.0117$) in the frequency of 7.5 and 8 Hz ($p < 0.05$) with respect to the vehicle group throughout the water maze test (Fig. 7, before the black arrow).

The inter-group analysis of coherence was conducted using an ANOVA for repeated measures considering the factor group and frequency as independent and the training days (1–6) as repetitions. The coherence between MS-DG ($F_{80,2315} = 1.486$, $P = 0.045$) and MS-CA1 ($F_{80,2315} = 1.302$, $P = 0.039$) showed significant effect for the interaction of frequency, group and day. When paired comparisons were made, the 5HT-D group showed a higher coherence on the day 2 in the 7.5 and 8 Hz frequencies ($p < 0.05$) in MS-DG and in the 7.5 Hz frequency in MS-CA1, compared with the vehicle group. The MS-SUM coherence shown a tendency in the interaction of factors, frequency, group and day ($F_{80,2390} = 1.236$, $P = 0.079$) and paired comparison showed a higher coherence for the 5HT-D group in the 7.5 Hz frequency on day 2 ($p < 0.01$) compared with the vehicle group. Similarly, the MS-MM coherence showed a tendency in the interaction of the factors, frequency, group and day ($F_{80,2390} = 1.272$, $P = 0.068$), paired comparison were made and the 5HT-D group showed a higher coherence in the 7.5–8 Hz frequencies ($p < 0.01$) on day 2 (Fig. 7, after the black arrow).

The coherence between regions during the baseline condition was not significantly different by day and the frequency in each group and between groups (data not shown).

4. Discussion

The functional role of serotonin on the medial septum and its impact on the theta activity that occurs during behaviours involving encoding and the retrieval of information as the spatial learning and hippocampus-dependent memory is poorly understood. The results of this study demonstrate that the reduction of septal serotonin induces a better performance in the initial stage of learning, such as shown in the distance travelled and the latency of the 5HT-D group (Fig. 2). Earlier distance reduction, latencies and reduced distances relative to the vehicle group since day 1, and an asymptotic level were reached faster by the experimental group. Additionally, a greater number of crosses on the site of the platform showing greater accuracy finding the platform, supporting the hypothesis that facilitation in the encoding of the information occurred during the first days of training in the 5HT-D group. Overall, these results suggest that there is greater efficiency in the processing of spatial information associated with a principal effect on the acquisition and retrieval of information.

The critical role of the medial septum in the regulation of the hippocampal function has been demonstrated [77,111]. The septal participation in both the acquisition and maintenance of spatial memory in the water maze task has been shown in that the aspiration or electrolytic abolition of the medial septum impaired these processes [41,34,13,30]. Intraseptal infusions of lidocaine disrupted the acquisition and retrieval of the task in the water maze (validated in [65]. Additionally, the role of septal cholinergic and GABAergic cells is important to establish spatial learning [88,87]. The application of intraseptal muscimol particularly impairs the acquisition of spatial information [16] and impairs long-term memory, suggesting that GABAergic regulation has an important role in septo-hippocampal functioning [86]. In addition, the relevant role of cholinergic cells in spatial learning has been demonstrated [31,46,45]. Ikonen et al. [49] suggested that the loss of cholinergic septo-hippocampal activity affects remapping, which is important for the establishment of hippocampal spatial representation for distinct environments. Similarly, Pang et al. [98] showed that the combined lesions on GABAergic and cholinergic septal cells produces an impairment of spatial memory in both the radial arm and the water mazes, although lesions of either cellular population alone do not impair or only mildly impair spatial memory. It has been suggested that the two types of septal cells are important for spatial learning and that lesions on cholinergic and GABAergic MS neurons produce profound effects on the hippocampal physiology [69,126].

The activity of GABAergic and cholinergic septal cells most likely of glutamatergic cells can be modulated by extrinsic afferents, particularly by serotonergic terminals originating in the median raphe neurons that synapse on these septo-hippocampal neurons, and possibly of terminals from the dorsal raphe neurons that innervate the medial septum, but not to the hippocampus [1] which express distributed serotonergic receptors types [82,27,1], including the 5HT1A, 5HT2A, 5HT2C and other receptors [72]. The facilitation of spatial information processing observed in the 5HT-D group was possibly due to changes in the serotonin action on these septal cells.

Although the participation of serotonin in spatial learning has been controversial, several studies have shown a subtle facilitation of learning after the depletion of serotonin, principally in the acquisition of the learning task in the Biel maze and Stone maze [100,2,89], as well as in inhibitory avoidance in mice [3]. Additionally, the cerebral depletion of serotonin was shown to have no effect

Table 2
Intra-group comparison of coherence between medial septum and hippocampus and between medial septum and mammillary nuclei.

Region/day		D1vsD2	D1vsD3	D1vsD4	D1vsD5	D1vsD6
MS-DG	Vehicle	ns	ns	ns	ns	9.5–10.5
	5HT-D	7.5	ns	ns	ns	8 and 9
MS-CA1	Vehicle	ns	ns	8.5–9	7.5–9	8–10
	5HT-D	7.5	ns	ns	ns	8 and 9.5
MS-SUM	Vehicle	ns	ns	8–9.5	8–9.5	8
	5HT-D	ns	ns	ns	ns	ns
MS-MM	Vehicle	ns	ns	8–9	8 and 9	8 and 9
	5HT-D	ns	ns	ns	ns	8 and 8.5

Comparisons between the day 1 and all other days of training (D1vs D2 to D6).
Frequency values in Hz. ns, not significant. $p < 0.05$.

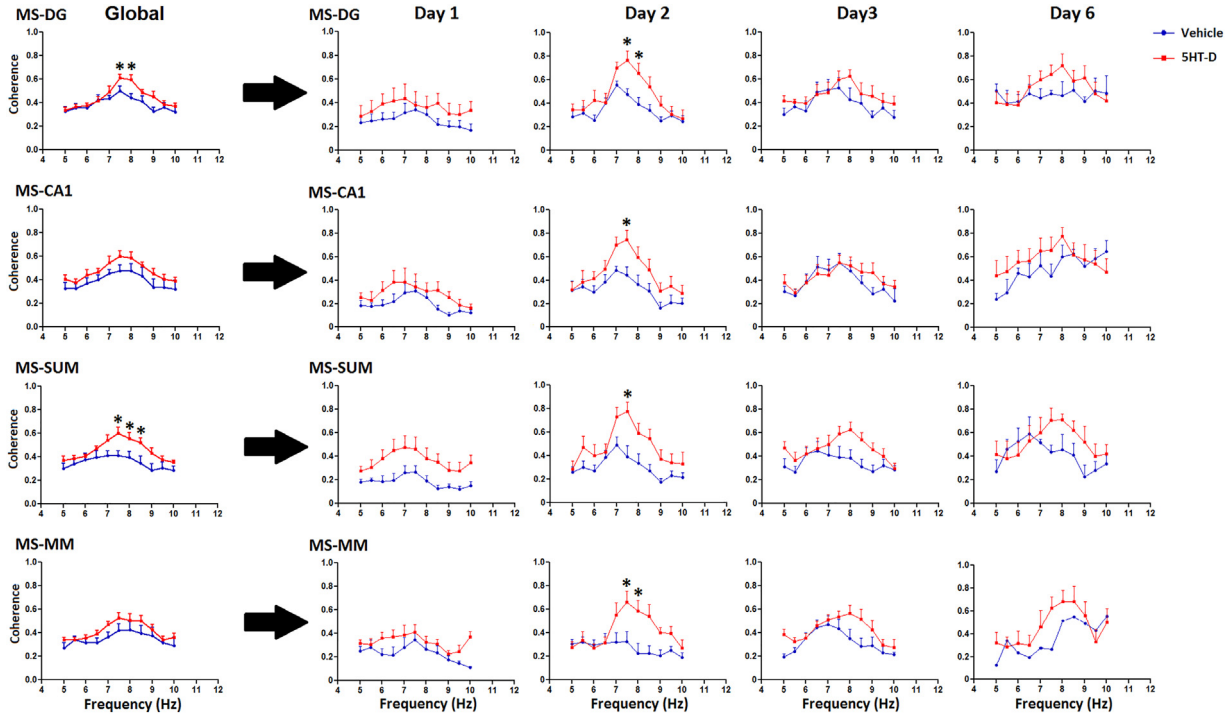

Fig. 7. The intergroup comparison of the coherence between the theta activity (4–12 Hz) recorded in the MS-DG, MS-CA1, MS-SUM, and MS-MM on each training day. Left: Coherence during the global performance on all days in each group (main effect). Right: After the arrow, the coherence on each training day during the search for the platform. Only days 1, 2, 3 and 6 are displayed. The coherence was different on day 2. Mean ± S.E.M. *, 5HT-D vs the vehicle. $p < 0.05$.

on learning evaluated in the Morris water maze and radial arm maze [85]. However, learning deficiencies caused by septal lesions (192 IgG-saporin) were reversed by specific hippocampal 5-HT depletion [70], which suggests that the attenuation of serotonergic tone in the hippocampus may compensate for some dysfunctions subsequent to the loss of cholinergic hippocampal inputs [70,103]. This observation is in close concordance with data showing that a reduction of the serotonergic tone, by the pharmacological activation of somatodendritic 5-HT1A receptors on raphe neurons, attenuates the cognitive disturbances produced by the intrahippocampal infusion of antimuscarinic drugs such as scopolamine [22,23]. In addition, the depletion of hippocampal serotonin led to the facilitation of spatial place learning in a water maze [39] and similarly, in this study, we observed that a depletion of septal serotonin facilitates spatial learning with a greater effect on the information acquisition phase; suggesting that serotonin in the medial septum plays a very important role in modulating the coding of information dependent of hippocampal function. However, however, it does not rule out a possible effect of 5,7-DHT on the serotoninergic fibres of the hippocampus.

In contrast, when 5-HT1A-receptor agonist 8-OH-DPAT is infused into the septum [52,9] or the hippocampus [24], or when it is systemically administered [26], the Morris water maze performances and working memory are impaired. Specifically, pre-acquisition intraseptal 8-OH-DPAT agonist infusions prevent learning in the water maze [66]. During this task, spatial memory encoding is altered and the receptor interferes with consolidation, but does not affect memory retrieval [65]. The effect of this receptor on acquisition could be mediated by non-cholinergic cells, such as GABAergic and glutamatergic cells, or through all types of cells [66] because the intraseptal administration of 8-OH-DPAT combined with a sub-threshold dose of the NMDA receptor antagonist (D-AP5) only marginally affected spatial acquisition, while it profoundly impairment spatial memory [33], suggesting possible serotonergic modulation of glutamatergic cells. In addition, the stimulation of 5-HT1A and 5HT1 B receptors induces poor performance in spatial learning [17,25].

Based on evidence from the previous studies mentioned above, the facilitation in information coding after septal serotonin reduction observed in this study could be mediated by serotonergic inhibitory receptors located on the septal cells. This would be in accordance with the proposal that the activation of cholinergic and GABAergic neurons is facilitated by a low serotonergic tone in the medial septum, making both encoding and consolidation easier,

although this result requires further investigations [53]. Also, this result could be explained by the fact that high levels of ACh activate circuits to facilitate the encoding of novel information and low levels of ACh may not be sufficient for encoding novel information, but the facilitate consolidation and retrieval of familiar information [46,45].

On the other hand, the observed changes in behaviour are associated with modifications of the RP and coherence at different frequencies of theta activity in the hippocampus, SUM and MM nuclei, during the first days of spatial learning. As animals improve its performance in spatial learning task, the power and frequency is increased, that is, a change occurs to the fast frequency (7.5–8 Hz) of hippocampal theta activity [40,47]. A similar effect was observed in the vehicle group where the RP of the fast frequency of theta activity from the hippocampus (the DG and CA1) was associated with reduced latency and distance travelled during the training days (since day 3). However, while both groups had increased frequencies throughout training, is noticeable that the 5HT-D group had a high frequency peak since day 1 (7.5–8 Hz), the switch to a high frequency occurred from day 2, and as the days progressed, the peak frequency increased to 8–9 Hz and was maintained until the last day of training. It has been demonstrated that a change in the hippocampal theta frequency can be functionally significant, a reduction of approximately 0.5 Hz was related with a modest impairment of spatial learning [96] and a specific frequency can be critical for hippocampal functioning [80,48]. It has been reported that a reduction of hippocampal theta frequency by environmental novelty (foraging) aids the encoding of new information into memory and the theta frequency increases with days of experience in such an environment [51]. In addition, in the present study changes in the specific frequency are associated with greater functional coupling between the medial septum with the hippocampus and mammillary nuclei, which was reflected in the greater coherence through learning in the 5HT-D group that principally occurred on day 2. This finding could indicate that the reduction of serotonin in the medial septum facilitates not only the communication of the medial septum with the hippocampus but also with the mammillary nuclei to facilitate information encoding, primarily during the initial phase of learning, as expressed in behaviour. A synchronization of theta activity during this time could help achieve an optimal level that is maintained until later days. Anatomically, the reciprocal communication of the medial septum with the hippocampus and mammillary nuclei [37,12,73] and collateral connection of the SUM with the hippocampus [118] has been reported; however, it is not possible to determine which region is influencing others in this study, i.e., it is unknown if the direct influence of the medial septum or hippocampus raised the changes in the power of the SUM. What is clear is that optimal communication dynamics in the septo-hippocampal and septo-mammillary circuit for efficient encoding, consolidation, and retrieval of information may be modulated by the effect of serotonin on septal cells. It has been shown that ibotenic acid lesions of the MS reduces the frequency of theta activity [74]. Additionally, it has been shown that the SUM is important to codifying hippocampal theta frequency in anesthetized and freely moving rats [125,97]. In turn, the medial mammillary nucleus integrates hippocampal descending information and sends it to the supramamillary core and is important for the encoding of spatial information and the modulation of hippocampal CA1 theta activity [64,62,113]. It has been shown that the blockade of medial septum with tetracaine does not affect rhythmicity in the SUM [80]; however, spontaneous slow theta in the SUM follows a theta drive of septal origin in anesthetized rats [63,61]. In the present study, the septal serotoninergic fibres were affected, and although changes in coherence trough days were not observed, the coherence was higher since day one, and notably higher functional coupling of MS with DG and SUM was observed as a global, which could indicate that the medial septum communication with these two regions is important in processing spatial information.

Previous studies shown that serotonin participates in modulating hippocampal theta activity in relation to information processing [94]. In particular, the serotonergic reduction in the hippocampus facilitated learning in the water maze, which was associated with higher relative theta activity power in the high frequency (6.5-9.5 Hz) in the CA1 [39]. A similar effect on RP was found in spatial working memory after reducing the septal serotonin content [76]. The present and previous studies support the hypothesis of the serotonergic system role as the desynchronizer of hippocampal theta activity, under which low serotonergic tone might allow theta activity and the codification of information [62,120]. However, the reduction of serotonin in the SUM/PH [40], or only in the SUM [47], produced learning deficiencies and the absence of theta activity learning-related changes in the CA1, and reduced communication between the MS and CA1, impairing the consolidation of the memory. The previous finding may indicate that serotonin act in the desynchronizing model, but only in relays of the system where the theta frequency information was already been encoded.

Serotonergic terminals from raphe neurons synapse on septo-hippocampal neurons whose activity can be modulated by serotonergic receptors [82]. It has been shown that serotonin released on the MS normally inhibits the rhythmic firing of septal cells, thereby producing hippocampal desynchronization [57]. Similarly, the participation of different serotonergic receptors in modulating theta activity has been observed, principally by the application of 5HT1A, 5HT2A and 5HT2C receptor antagonists that produce a hippocampal theta rhythm and enhance theta oscillation in the MS, while in contrast, the agonist administration inhibited the firing activity of the recorded MS neurons and suppressed the MS and hippocampal theta rhythm [55,56,42,109]. It is likely that the septal serotonin depletion disinhibited the MS neurons and thus facilitated theta-associated learning, indicating a tonic regulation of the septo-hippocampal system by serotonin.

In contrast to the analysis of RP and coherence during the search condition of the platform, the RP was determined in the baseline condition (awake-quiet) before the start of training in the maintenance cage on different days. First, it was noticeable that the RP of the MS, DG, CA1, SUM, and MM regions in the baseline condition did not change over the days of training in the vehicle and 5HT-D groups; however, the intergroup analysis showed that the 5HT-D group presented with lower RP in the 6.5 and 7 Hz frequencies only in the DG of hippocampus nearly every day. In relation of this data, two types of hippocampal theta have been distinguished by their pharmacology and behaviour. Theta type 1 (atropine-resistant) is associated with voluntary movement and exploratory behaviours (walking, rearing, running, visual exploration, and postural changes), whereas theta type 2 (atropine-sensitive) is characterized during immobility (reflexive behaviours, grooming, and eating) and during urethane anaesthesia [112,67]. We suggest that despite the fact that type 2 (immobility) is dependent on the cholinergic system, the decrease of the RP of the theta in the 5HT-D group with respect to the vehicle group could have a serotonergic component, which could be associated with the preparatory state of movement [112]. Recently, the activation of the medial septum has been shown to generate a atropine-sensitive and atropine-resistant hippocampal theta rhythm [38] in vitro, and the participation of GABAergic septal cells is important to exploratory behaviours and type 2 theta [35].

It has been reported that theta activity is associated with movement and running speed, and can change their power and frequency [78,84,108]: the faster an animal runs, the higher the theta frequency; however, the motor activity displayed by the two groups was similar and had no significant differences in velocity during the days of training (Fig. 2C) and the correlation of velocity

with the theta frequency peak of DG and CA1 was not significant in either group (Fig. 6B). This finding indicates that the changes observed in the frequency of theta are more possibly associated with the cognitive process without discarding sensorimotor information processing, which is also being integrated [5,11,102]. In support of this association, changes in the frequency only in the task of place learning but not cue-learning training [92], and an increase of hippocampal theta oscillations during the cognitive process of spatial decision making has been shown, discarding the velocity [8].

Finally, this study demonstrated that the septal serotonin depletion facilitates the encoding of spatial information during the first days of training in association with changes in the high frequency theta activity and a higher functional coupling of the medial septum with hippocampus and mammillary nuclei. This finding is in accordance with the role of serotonin in modulating the hippocampal theta function and learning.

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Supramammillary serotonin reduction alters place learning and concomitant hippocampal, septal, and supramammillar theta activity in a Morris water maze

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Hippocampal theta activity is related to spatial information processing, and high-frequency theta activity, in particular, has been linked to efficient spatial memory performance. Theta activity is regulated by the synchronizing ascending system (SAS), which includes mesencephalic and diencephalic relays. The supramamillary nucleus (SUMn) is located between the *reticularis pontis oralis* and the medial septum (MS), in close relation with the posterior hypothalamic nucleus (PHn), all of which are part of this ascending system. It has been proposed that the SUMn plays a role in the modulation of hippocampal theta-frequency; this could occur through direct connections between the SUMn and the hippocampus or through the influence of the SUMn on the MS. Serotonergic raphe neurons prominently innervate the hippocampus and several components of the SAS, including the SUMn. Serotonin desynchronizes hippocampal theta activity, and it has been proposed that serotonin may regulate learning through the modulation of hippocampal synchrony. In agreement with this hypothesis, serotonin depletion in the SUMn/PHn results in deficient spatial learning and alterations in CA1 theta activity-related learning in a Morris water maze. Because it has been reported that SUMn inactivation with lidocaine impairs the consolidation of reference memory, we asked whether changes in hippocampal theta activity related to learning would occur through serotonin depletion in the SUMn, together with deficiencies in memory. We infused 5,7-DHT bilaterally into the SUMn in rats and evaluated place learning in the standard Morris water maze task. Hippocampal (CA1 and dentate gyrus), septal and SUMn EEG were recorded during training of the test. The EEG power in each region and the coherence between the different regions were evaluated. Serotonin depletion in the SUMn induced deficient spatial learning and altered the expression of hippocampal high-frequency theta activity. These results provide evidence in support of a role for serotonin as a modulator of hippocampal learning, acting through changes in the synchronicity evoked in several relays of the SAS.

Keywords: supramammillary nucleus, serotonin, septum, hippocampus, theta activity, spatial learning

INTRODUCTION

Hippocampal theta activity has been related to processing of spatial information in different behavioral paradigms in various animal species (Ammassari-Teule et al., 1991; McNaughton et al., 2006) as well as in human beings (Klimesch et al., 1994; Klimesch, 1999; Caplan et al., 2001; Ekstrom et al., 2005; Lega et al., 2014). The relation of theta activity and place learning has been also studied; changes in power and/or frequency of the hippocampal theta activity have been associated with efficient learning during place learning tests in the Morris maze (Pan and McNaughton, 1997; Olvera-Cortes et al., 2002, 2004; Olvera-Cortés et al., 2012; Buzsaki, 2005; Ruan et al., 2011), conditioning (Berry and Seager, 2001; Berry and Hoffmann, 2011), working memory (Mitchell et al., 1982), and novelty detection (Aggleton and Brown, 1999; Vinogradova, 2001), among others. Moreover, deficient spatial memory has been observed after the reductions in the frequency of hippocampal theta activity (Winson, 1978; Pan and McNaughton, 1997).

Theta activity is modulated by a group of mesencephalic-diencephalic structures called the synchronizing ascending system (SAS) (Bland et al., 1990; Kirk et al., 1996; Leranth et al., 1999; Woodnorth et al., 2003). Theta activity can be generated in the hippocampus by stimulation of the nucleus *reticularis pontis oralis* (RPOn) both in anesthetized and in awake animals (Vertes, 1982, 1986). It was proposed that the RPOn theta modulation spreads through the tegmental pedunculopontine nucleus (TPPn) to the hypothalamic relays, the supramammillary (SUMn) and posterior hypothalamic (PHn) nuclei (Takano and Hanada, 2009). Because of to the tonic firing of RPOn neurons, the rhythmical firing of SUMn cells, and the result from inactivating SUMn, it was proposed that SUMn convert the tonic input received from the RPOn into a rhythmical pattern, which is relayed to the medial septum (MS), considered the pacemarker of the theta activity (Gogolak et al., 1968; Petsche et al., 1968; Andersen et al., 1979; Kirk and McNaughton, 1991; Kirk and Mackay, 2003). In support of this hypothesis, procaine infusions into (medial) SUMn induce a decrease in the frequency of hippocampal theta activity elicited by stimulation of RPOn in awake or in anesthetized rats (Kirk and McNaughton, 1993; McNaughton et al., 1995). Moreover, the rhythmic activity in the SUMn elicited by infusing carbacol into the RPOn persists after either the infusion of procaine into the MS or the bilateral transection of the communication pathways between SUMn and the MS (Kirk et al., 1996; Kirk, 1997). Additionally, an efferent influence from MS, which induce the deceleration of theta frequency-related firing in SUMn neurons, was observed (Kocsis, 2006; Kocsis and Kaminski, 2006); this influence could originate in the reciprocal connections between the two nuclei (Vertes, 1992), possibly through a GABAergic, input from the lateral septum (LS) on the (lateral) SUMn (Leranth and Kiss, 1996).

The SUMn has been related to information processing in memory. SUMn c-fos activity increases in spatial tasks (exploration, reference memory, and working memory) in the Morris water maze (Santin et al., 2003). Additionally, SUMn inactivation through the micro infusion of TTX induces deficiencies in reference memory retrieval (when TTX is applied in the seventh day of training, but not in the fourth day of training) and deficiencies in spatial working memory (Aranda et al., 2008). Furthermore, inactivation of SUMn with lidocaine impairs memory retrieval and consolidation in spatial memory tasks (Shahidi et al., 2004a). These results remarkably suggest that the SUMn functions in spatial information processing, although a relationship between SUMn and spatial learning is less clear (Santin et al., 2003). One study explored the relation between the SUMn, hippocampal theta activity and learning. Infusion of cholrdiazepoxide (CDP) into the (medial) SUMn had modest effects on theta activity and place learning in Morris water maze (Pan and McNaughton, 1997). However, after lidocaine inactivation of MS and the concomitant lack of hippocampal theta activity, both place learning and the rhythmicity of hippocampal theta activity (7.7 Hz) were restored by using the SUMn oscillation to rhythmically stimulate the fornix (McNaughton et al., 2006). This study demonstrated the relevance of both the SUMn and theta activity for place learning.

Similarly to the other relay nuclei of the SAS and the hippocampus, the SUM receives serotonergic axons both from medial and dorsal raphe nuclei (Vertes, 1988, 1992). The role of the serotonin originated in the raphe nuclei in desynchronizing of the hippocampal EEG is well documented. Briefly, stimulation of the medial raphe nucleus (MRn) desynchronizes the hippocampal EEG through the action of serotonin, whereas the electrolytic lesions of the same nucleus induce hippocampal EEG with a higher magnitude and longer duration, which is also present during immobility, in rats (Assaf and Miller, 1978; Maru et al., 1979). Furthermore, mucimol, buspirone and 8-hydroxy-2-(di-*n*-propyl-amino)-tetralin (8-OH-DPAT), a 5-$HT1_A$ agonist, injections in MRn, induce persistent theta activity in the hippocampus of anesthetized rats, through the inhibition of serotonergic neurons (Vertes et al., 1994; Kinney et al., 1995). Thus, the serotonin can act on the SAS through many relays, or directly on the hippocampus to regulate theta activity; as a negative regulator of theta rhythmicity, serotonin could contribute to the fine-tuning of theta activity in the SUMn and thus influence on the upper relays, principally the MS and the hippocampus.

The role of serotonin as a modulator of learning has been extensively studied, although a complex picture emerges from the various papers possibly due to differences in learning tasks as well as differences in experimental strategies to manipulate the cerebral or regional serotonin activity, because of these factors, impairment, no effect or improvement in learning tasks has been reported after serotonin manipulations. Impairment in water maze tests was observed both after intra-septal or intra-hippocampal infusions of 8-OH-DPAT (Carli et al., 1992; Carli and Samanin, 1992; Bertrand et al., 2000). It has also been reported that intra-septal infusion of 8-OH-DPAT causes deficient spatial working memory (Jeltsch et al., 2004). In contrast, improvement in working memory and conditioning as well as improvement in place learning has been reported after reductions in cerebral, prefrontal and hippocampal serotonin (Altman et al., 1989; Pérez-Vega et al., 2000; Sarihi et al., 2000; Gutiérrez-Guzman et al., 2011). Additionally, a relation

between the serotonergic modulation of theta and hippocampal-dependent place learning has been found (Gutiérrez-Guzman et al., 2011; Lopez-Vazquez et al., 2014). Moreover, reduction of serotonin content in the SUMn/PHn induced place learning deficiencies associated with a lack of learning-related increases in high-frequency hippocampal theta activity through the training (Gutiérrez-Guzman et al., 2012). Thus, the SUMn is a relay of the SAS participating in the modulation of hippocampal theta activity, and it is at least partially involved in place learning consolidation and/or recovery; it also receive serotonergic inputs, which could modulate the fine-tuning of hippocampal theta activity. However, despite the above, the effects of serotonin SUMn depletion alone on both spatial learning and on the characteristics of hippocampal, septal and SUMn theta activity during place learning have not been evaluated. The aim of the present work was to evaluate the consequences of serotonin depletion in the SUMn on place learning and the concomitant theta activity recorded from the SUMn, medial septum (MS), dentate gyrus (DG), and CA1, during the training in the Morris maze, in the rat.

METHODS

Animals

Seventeen male, 4-months-old Sprague Dawley rats were used. The rats were maintained under standard facility conditions, and all of the experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and for the "Norma Oficiál Mexicana" for the use of experimental animals (NOM-062-ZOO-1999). All of the experiments were and approved by the Research Ethics Committee of the Instituto Mexicano del Seguro Social.

Surgery

The rats were divided in two groups, one control group (CTR, $n = 7$), and one experimental group (EXP, $n = 10$). Both groups of rats were anesthetized under ketamine/pentobarbital anesthesia (60 mg/kg im, 20 mg/kg ip) and chronically implanted with bipolar, concentric electrodes in the MS (coordinates: 0.6 mm anterior to the bregma, 1.5 mm lateral to the midline, 15° from the vertical, and 6.8 mm ventral to the cranial surface), DG (coordinates 3.5 mm posterior to the bregma, 1.5 mm lateral to the midline, and 3.4 mm ventral to the cranial surface), CA1 (coordinates: 4.5 mm posterior to the bregma, 2.4 mm lateral to the midline, and 2.7 mm ventral to the cranial surface), and SUM (coordinates: 4.7 mm posterior to bregma, 0.2 mm lateral to the midline, and 8.7 mm ventral to the cranial surface); all coordinates were taken from the Atlas of Paxinos and Watson (1998). The electrodes were made of nichrome wire with a diameter of 60 μm fastened inside a stainless steel # 27 caliber cannula isolated with epoxy resin, with a small surface exposed at the tip. The electrodes were fixed to the skull with dental acrylic. Two screws were used, one placed in the frontal bone served as ground and the other placed in the posterior skull served to fix the implant. In the same surgery, rats in the EXP group received an intra-SUM infusion of 5 μg of 5,7-DHT (2 μg dissolved in 0.1 μl of 0.1% ascorbic acid in saline solution) at an infusion rate of 0.1 μl/min for 4 min. One injection was placed into the SUMn (4.7 mm posterior to the bregma, 0.2 mm lateral to the midline, and 8.24 mm ventral from the cranial surface) using a Hamilton syringe and an infusion pump. Thirty minutes before the 5-HT lesion, the rats received desipramine (30 mg/kg, ip) to protect the noradrenergic terminals. The rats of the CTR group only received an infusion of vehicle solution, similar in volume and rate to the EXP group.

Behavioral Test

Two weeks after the surgery, the rats were trained in a place-learning test using the Morris water maze. This maze consisted of a circular pool (1.5 m of diameter and 45 cm of height wall) filled with water made blue by adding gentian violet, which contained a submerged circular platform (9 cm of diameter) placed in a fixed position in one of the four virtual quadrants of the maze.

The rats were submitted to four daily trials during six consecutive days; each trial was initiated by placing the rat into the pool facing the wall in one of the quadrants (the starting quadrants were randomly chosen each day but were similar for all rats in one day). The trial continued until either the rat located the platform or 60 s elapsed. If the rat failed to locate the platform in this time, it was guided to the platform by the experimenter and left there for 15 s. After this time, the rat was retired and placed in a home cage during 2 min (inter-trial period) before beginning the next trial. On the seventh day, all rats received one 30 s probe trial that consisted of searching the maze after the escape platform had been removed. The behavioral tests were video recorded and stored on a computer for later analysis, when the escape latencies, distances traveled and swimming velocity achieved by the rats and also the distance swam for each quadrant in the probe trial were obtained. Recordings and analysis were performed using the Data-Wave Inc. software (VideoBench 5.1). The mean swim distances from the four daily trials as well as the mean daily latencies were compared. In the probe trial, the distance swam by the rats in each quadrant was obtained and compared.

EEG Records

Each training day the rats were connected to a commutator (Neuro-Tek, CA. IT,) using a cable with a male connector. The commutator was connected to one amplifier (Neurodata acquisition system, GRASS Mod 15, Astro Med Inc. 600 E. Greenwich Ave., W. Warwick, RI 02893, USA) and the EEG was digitalized to 1024 Hz with a DataWave Technologies data acquisition system, and the EEG was stored in a PC to be analyzed of line. A bipolar recording was taken using the nichrome wire as G1 and the cannula as G2 (A bipolar derivation was made using the G1–G2), the filters were set to 1–100 Hz, the EEG recording were synchronized to the VideoBench software, which tracked a small light-emitting diode attached to animal implant. A baseline recording was taken from the awake-immobile rat in the cage (60 s), and then, all time that the rat searched for the platform was recorded, including the final 15 s that the rats remained onto the escape platform. The data were imported into MATLAB

(Mathworks, Inc.) (Delorme and Makeig, 2004) and the software EEGLAB was used to eliminate artifact by visual inspection.

The EEG from basal and searching conditions was submitted to the Fast Fourier Transform (FFT) and absolute power was obtained as the mean spectrum of 2-s samples, to ensure a resolution of 0.5 Hz, from 4 to 12 Hz. The relative power (RP) was obtained for each behavioral condition and 0.5 Hz of frequency as the percent of the total 4–12 Hz absolute power band. Comparisons were made of the RP in the range of 5–0 Hz, in each brain region, between days and frequency for each group (intra-group comparisons) and between day, group and frequency (inter-group comparison); using an ANOVA for repeated measures and paired *t*-test with a Bonferroni correction. Additionally, coherence values were computed for pairs of recording sites and compared in manner similar to the RP values. The analyses of both EEG power and coherence were conducted using custom programs adapted from Ken's MATLAB library written by Ken Harris and available at http://osiris.rutgers.edu/Buzsaki/software.

HPLC

The serotonin content was determined using HPLC as follows, after the euthanasia of the animals, samples including SUMn were dissected from a slice containing the region of interest and a sample of the tissue was punched using a 25 G cannula. The tissue samples were homogenized in 1N HCl and centrifuged. The content of serotonin and 5HIAA (pg/mg of fresh tissue) of the supernatant was determined using a LiChroCart purospher star column (150 – 4.6, RP – 18 end caped, 5 mm, MERK KGa A, Darmstadt; Germany) with a mobile phase (pH 3.1) composed of citric acid (50 mM), H_3PO_4 (50 mM), EDTA (20 mg), octanesulfonic acid (120 mg/L), and methanol (8 %). The flow rate was 1.3 mL/min. An electrochemical detector (AtecLydenVT-03) with a work potential of 0.800 mV adjusted to the pH of the mobile phase was used. The data were compared using the Student *t*-test.

The SUMn was visually inspected to verify the electrode position, during the dissection of the tissue for HPLC. The tract of the electrode in the remaining tissue after the dissection of SUMn for HPLC and the position of the other electrodes was verified using a light microscope after the brain was sliced at 5 μm and the slices were stained with cressyl violet (**Figure 1**). After histological verification of the position of the electrodes in the MS, the DG and the CA1; the EXP group of rats included only those rats with reductions of serotonin greater than 50% from the CTR group mean content in the SUMn; thus, four rats were excluded because they showed a reduction of serotonin less than 50%, and the EXP group included 6 rats in the final analysis.

RESULTS

Serotonin Content

The EXP group had significantly lower serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations that the CTR group (Paired one tailed $t = 4.274$, $df = 5$, $p = 0.004$ and $t = 7.293$, $p = 0.0004$, $df = 5$; for 5-HT and 5HIAA, respectively) (**Figure 2A**).

Behavior

Escape latencies were compared between training days within the two groups of animals using a Friedman ANOVA, and a *post-hoc* Wilcoxon test. The CTR group significantly reduced their escape latencies (X^2_r= 23.245, $P > 0.001$), by day 3–6 ($p = 0.018$); whereas the EXP group only significantly reduced their escape latencies (X^2_r = 11.429, $P = 0.044$), on day 6 ($p = 0.028$). Intergroup comparisons (Mann Whitney U test) showed both a main effect ($\sum R_x = 391$, $P < 0.001$) and differences in the escape latencies on days 3 ($p = 0.004$), and 5 ($p = 0.003$) with a bias toward day 4 ($p = 0.063$); the escape latencies were longer for the EXP than for the CTR group (Data not showed).

Intra-group comparisons of the distances traveled by the rats were made using an ANOVA for blocks and Tukey *post-hoc*; the CTR group significantly reduced their distances traveled [$F_{(5, 30)}$ 24.112, $p < 0.001$], on days three to six of training ($p < 0.001$). The EXP group did not show significant reduction of distance traveled over the training days [$F_{(5, 25)} = 2.018$, $p = 0.111$]. Inter-group comparisons using two factors, group and day of training, were made using an ANOVA for repeated measures. The distances traveled by the EXP group were higher than the distances traveled by the CTR group [$F_{(1, 11)} = 11.232$, $p = 0.006$, main effect], however, there was no significant interaction of day and group [$F_{(5, 55)} = 2.242$, $p = 0.062$] (**Figure 2B**). The swimming velocities were compared similarly to the distances, but no changes over the training days were observed for the CTR [$F_{(5, 30)} = 1.472$, $P = 0.228$] or the EXP [$F_{(5, 25)} = 1.981$, $P = 0.116$] groups (**Figure 2D**).

Finally, the distance traveled in each quadrant during the probe trial (day seven) was compared between quadrants and groups, using a Two-Way ANOVA (group and quadrant). No significant differences between groups were observed [$F_{(3, 48)} = 2.452$, $P = 0.074$]. However, the CTR group swam significantly different distances between quadrants [$F_{(3, 24)} = 6.285$, $p = 0.002$]; the distance on the quadrant that had contained the platform in the training (N) was higher than in the S and W quadrants. The EXP group of animals swam similar distances in all quadrants (**Figure 2C**).

Theta Activity

The row EEG from the four regions recorded, under basal conditions (awake, immobile, wet rat) in the cage and during the searching for the platform on days one and six of representative rats, is shown in **Figure 3**. The natural logarithm (nl) of the absolute power of the theta band from each cerebral region and group was compared by day and frequency using Two-Way ANOVA. No significant differences were observed in any group regarding this comparison (data not shown). Intergroup comparisons of the absolute power of the nl recorded from each cerebral region were performed using ANOVA for repeated measures of two factors (group and frequency), with days as a repeated measure; no significant differences were observed for any of the regions studied.

Relative power, expressed as a percentage of the contribution of each specific frequency to the total power of the theta band, had a beneficial effect of reducing the inter-subject variance. In addition, it is possible that the changes associated with learning

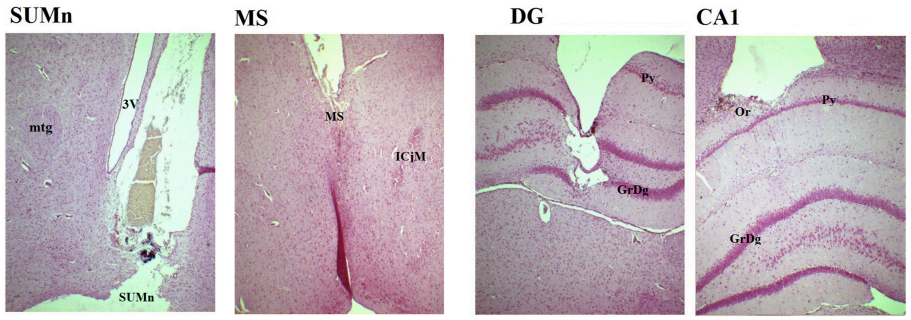

FIGURE 1 | Photomicrography of representative slices showing the position of the electrodes in the four regions. In the SUMn note that tract of the electrode reaches the zone in which the tissue was punched out for HPLC measures. SUMn, supramammillary nucleus; mtg, mamillotegmental tract; 3V, 3rd ventricle; MS, medial septum; ICjM, Major island of Calleja; DG, dentate gyrus; GrDg, granular layer of dentate gyrus; Py, pyramidal layer of CA1; CA1, field CA1 of hippocampus; Or oriens layer. Magnification 4X.

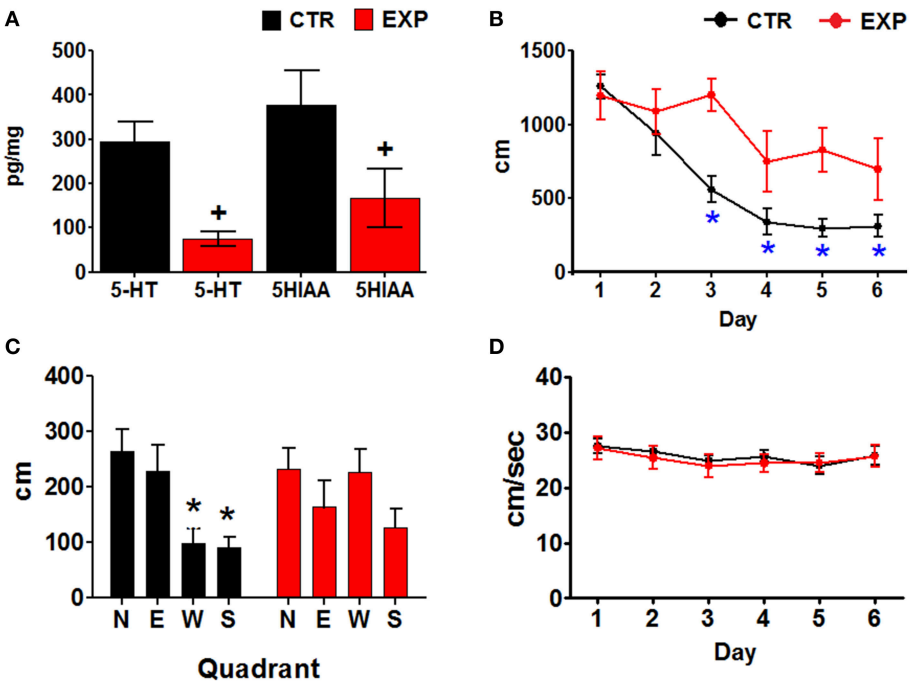

FIGURE 2 | (A) Serotonin and 5-HIAA concentrations. Mean ± SEM, $p < 0.05$. **(B)** Distances traveled by the two groups of animals, through the training days. Mean ± SEM. *Day 1 vs. subsequent days, $p < 0.001$. **(C)** Distance traveled in each quadrant during the probe test of the two groups of rats. The escape platform was placed in the north quadrant during the training. North (N), South (S), East (E), and West (W) quadrants. Mean ± SEM. *N quadrant vs. all other quadrants; +, group CTR vs. EXP, $p < 0.05$. **(D)** Swimming velocities displayed by the two groups of animals through the training days. Mean ± SEM.

on EEG could be sufficiently subtle to reflect absolute power changes; moreover, the consequences of a reduction of one neurotransmitter in one discrete nucleus from the SAS could be quite subtle and could induce changes in the expression of absolute power in the theta band. Thus, more subtle changes were expected than those observed in studies in which the cerebral reduction of serotonin or RM lesions was induced. Using this rationale, in previous studies, learning-related changes were observed in the relative power of the theta activity recorded in CA1 during the training of rats in the Morris water maze (8–10).

The relative power (RP) of each cerebral region of the CTR group was compared by day and frequency using a Two-Way ANOVA for repeated measures. The RP recorded in the SUMn for the CTR group changed across the training days [$F_{(50,\ 330)} = 2.977$, $p < 0.0001$]. The RP for high frequencies (7.5–8.5 Hz) RP increased with the training days whereas low frequencies (6.5 and 7 Hz) decreased when compared with the first and second days. RP from MS showed significant changes across the training days [$F_{(50,\ 330)} = 1.477$, $p = 0.025$]; particularly an increase for the 8 Hz frequency the lasts days of training. The RP from

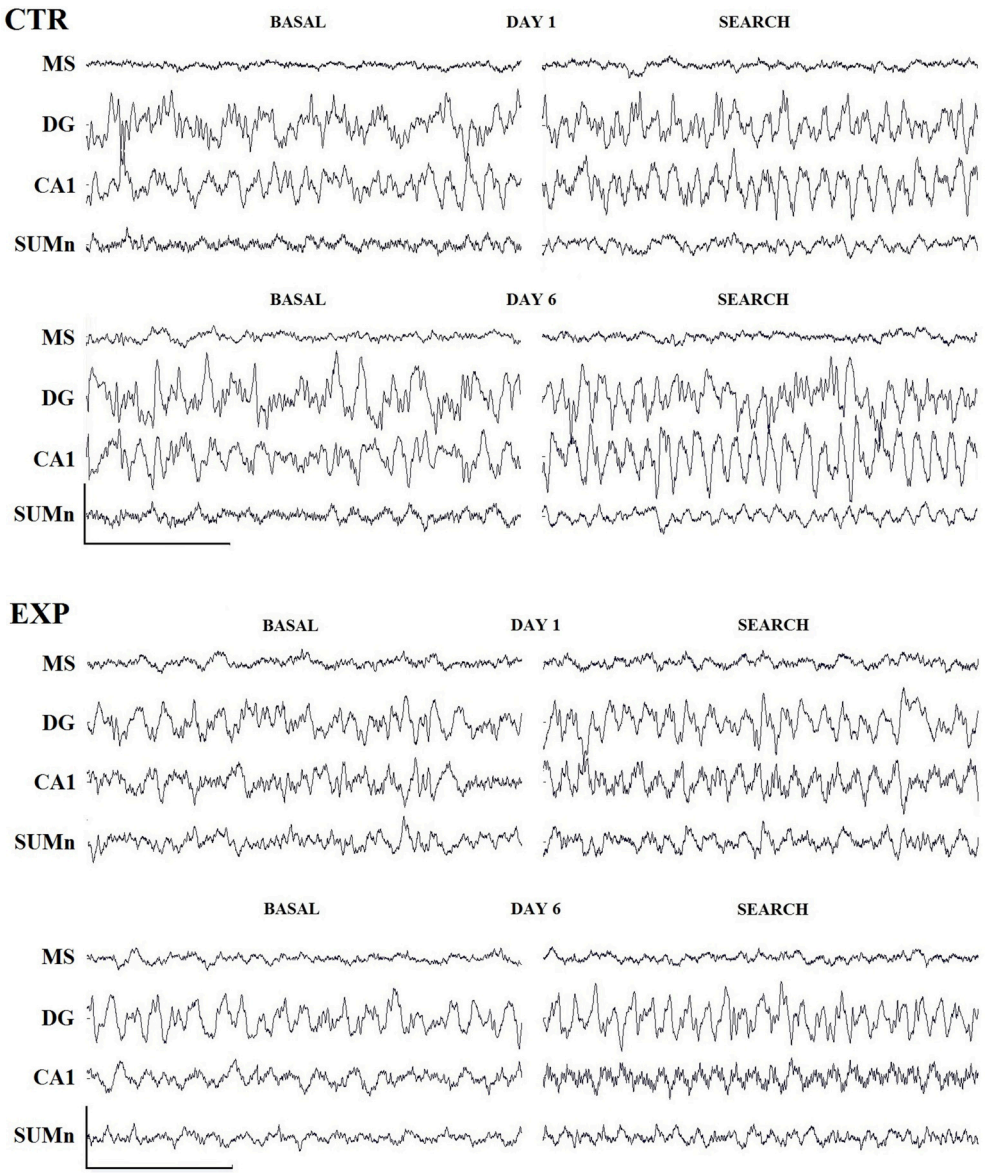

FIGURE 3 | Row traces of one representative rat from each group. Three second samples recorded during basal conditions (awake, wet rat in a holding cage) and during the search for the platform on days 1 and 6 of training. Cal. 500 mV/1 s. Abbreviations are as in the text.

the DG showed increased theta activity over the course of the training days [$F_{(50,\ 330)} = 2.689$, $p < 0.0001$] for the 7–8.5 Hz frequencies. Finally, the RP of the theta activity recorded in the CA1 showed changes with regard to training days [$F_{(50,\ 30)} = 2.729$, $p < 0.0001$], and the RP for the 6.5 and 7 Hz frequencies was reduced, whereas the RP for 8.0 and 8.5 Hz increased over the training days. **Figure 4** shows the RP only for days 1, 2, 5, and 6 when the differences between RP were maximal, and **Table 1** shows the significant differences between all of the training days from the four regions. Thus, the RP in the higher frequencies (7–5–10 Hz) increased across the training days in the different regions, and some regions showed a concomitantly reduction in low frequencies RP (6.5–7 Hz).

The RP recorded in the four cerebral regions from the EXP group did not show significant effects of training across training days [$F_{(50,\ 275)} = 1.272$, $p = 0.1181$ for MS; $F_{(50,\ 275)} = 1.010$, $p = 0.4622$ for DG; $F_{(50,\ 275)} = 1.272$, $p = 0.1178$; and $F_{(50,\ 275)} = 1.368$, $P = 0.0616$ for SUMn]. However, there were days in which the information processing was putatively different, that is, acquisition of information is prominent on days 1 and 2, whereas the consolidation and recovery of memory is prominent on days 5 and 6; therefore, an ANOVA including only days 1, 2, 5, and 6 for the EXP group was performed to determine whether the differences in processing would be expressed as differences in EEG in this group. The SUMn RP showed significant changes across training days when only the

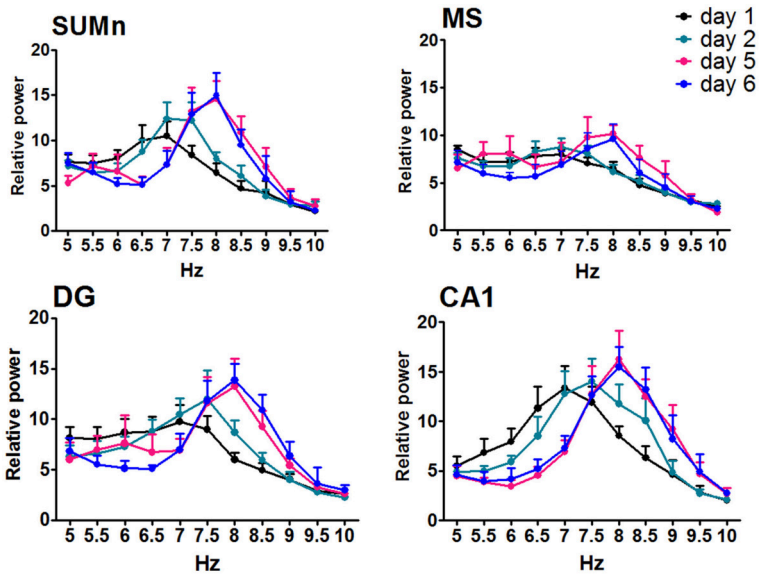

FIGURE 4 | Relative power in the theta band (5–10 Hz) recorded in the four regions of the CTR group during the searching of the platform across days of training. Only days 1, 2, and 5, 6 are showed. Mean ± SEM. Significant differences are listed in **Table 1**. $p < 0.05$.

TABLE 1 | Comparison between training days of the relative power recorded during the searching for the platform in the Morris water maze task in the CTR group.

Hz\Day	1	2	3	4	5	6	Region
6.5	10.00 ± 1.71	8.74 ± 1.32	6.97 ± 0.92	6.78 ± 0.97	5.16 ± 0.85^{A}	5.11 ± 0.85^{A}	
7	10.49 ± 1.62	12.40 ± 1.85	11.25 ± 1.98	10.17 ± 2.13	7.30 ± 1.84^{B}	7.31 ± 1.53^{B}	
7.5	8.40 ± 0.89	12.2 ± 2.02	14.23 ± 2.30^{A}	13.98 ± 3.21^{A}	13.23 ± 2.59^{A}	12.87 ± 2.40^{A}	SUM
8	6.45 ± 1.00	7.98 ± 0.73	10.19 ± 0.96	11.73 ± 1.67^{A}	14.57 ± 1.97^{ABC}	14.96 ± 2.47^{ABC}	
8.5	4.70 ± 0.84	6.07 ± 1.15	7.02 ± 1.70	8.55 ± 2.30	10.86 ± 1.80^{AB}	9.48 ± 1.74^{A}	
8	6.50 ± 0.72	6.13 ± 0.73	6.23 ± 0.86	7.39 ± 0.60	10.13 ± 0.91^{A}	9.62 ± 1.54^{A}	MS
7.5	11.90 ± 1.59	14.04 ± 2.29	16.47 ± 3.02^{A}	13.08 ± 3.09	12.73 ± 2.84	12.60 ± 1.91	
8	8.51 ± 0.97	11.76 ± 1.95	12.64 ± 2.73^{A}	10.62 ± 1.57	16.27 ± 2.87^{A}	15.48 ± 2.01^{ABCD}	DG
8.5	6.32 ± 1.20	10.05 ± 2.23	9.01 ± 2.26	8.43 ± 2.15	12.51 ± 1.74^{A}	13.20 ± 2.20^{ABD}	
6.5	11.32 ± 2.17	8.49 ± 1.96	6.43 ± 1.36	6.66 ± 1.28	4.55 ± 0.89	5.19 ± 0.96^{A}	
7	13.36 ± 2.25	12.75 ± 2.33	12.15 ± 2.40	9.74 ± 1.68	6.93 ± 1.13^{BC}	7.29 ± 1.24^{AB}	CA1
8	8.51 ± 0.97	11.76 ± 1.95	12.64 ± 2.73	10.62 ± 1.57	16.27 ± 2.87^{AD}	15.48 ± 2.012^{A}	
8.5	6.32 ± 1.20	10.05 ± 2.23	9.01 ± 2.26	8.43 ± 2.15	12.51 ± 1.74^{A}	13.20 ± 2.20^{A}	

ANOVA including the 6 days of training was significant. Values are the mean ± SEM. A, B, C, and D show significant differences compared with days 1, 2, 3 and 4, respectively. P < 0.05.

mentioned days were considered [$F_{(30,\ 165)} = 2.194, P = 0.0009$]; with increases in the RP for the 7.5 and 8 Hz frequencies. In addition, in the CA1 region, the RP showed significant changes across days [$F_{(30,\ 165)} = 1.750, p = 0.0146$] for the frequencies 6.5, 7.5, and 8 Hz (**Figure 5**), the **Table 2** shows the significant differences in the two regions. In summary, the EXP group had minimal changes related to the process of leaning evident only when the comparisons included only the days 1, 2, 5, and 6. Moreover, the increased RP observed was limited to SUM and CA1 and occurred at 7.5 and 8 Hz, whereas no change was evident in this group at 8.5 Hz.

The mean peak frequency of each day of training from the four daily trials was obtained, and intra-group comparisons were made using ANOVA for blocks. The CTR group significantly increased the peak frequency in the SUMn [$F_{(5,\ 30)} = 60.061$, $p < 0.001$]; however, paired comparisons (Tukey's test) did not show significant differences compared with day 1. Additionally, the peak frequency in the DG increased with the day of training [$F_{(5,\ 30)} = 4.611$, $p = 0.003$]; the peak frequency increased on days 5 ($p = 0.004$) and 6 ($p = 0.034$) compared with the first day of training. The EXP group did not show increase in the peak frequency across training days in any region. Finally,

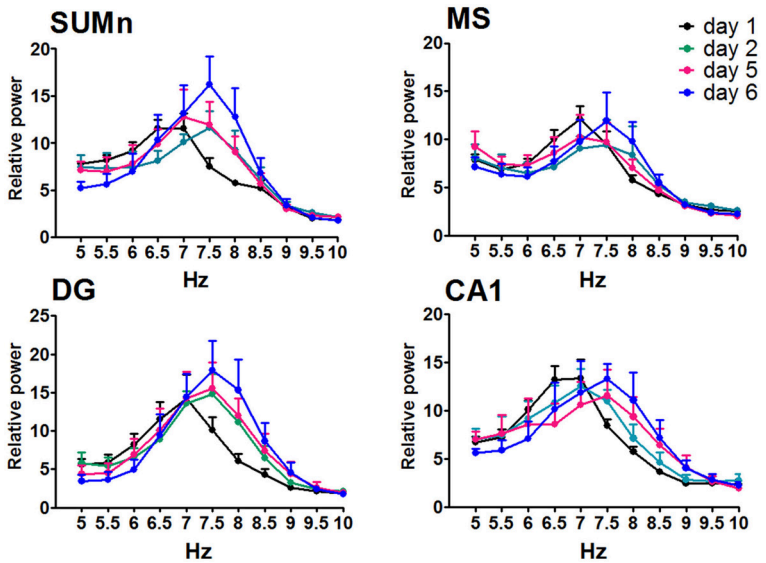

FIGURE 5 | Relative power in the theta band (5–10 Hz) recorded in the four cerebral regions of the EXP group during the searching of the platform across training days. The ANOVA was significant only when compared the days 1, 2, 5, and 6. Mean ± SEM. Significant differences are listed in **Table 2**. $p < 0.05$.

TABLE 2 | Comparison between training days of the relative power recorded during the searching for the platform in the Morris water maze task in the EXP group.

Hz\Day	1	2	3	4	5	6	Region
7.5	7.49 ± 0.93	11.60 ± 1.81	12.96 ± 2.20	10.18 ± 1.75	11.92 ± 2.47	16.25 ± 2.96^{AB}	SUM
8	6.72 ± 0.32	9.28 ± 2.07	9.35 ± 1.84	9.31 ± 2.80	9.01 ± 1.71	12.81 ± 3.01^{A}	
6.5	13.22 ± 1.45	10.86 ± 1.76	10.88 ± 1.94	9.09 ± 2.50	8.60 ± 2.15^{A}	10.18 ± 2.75	
7.5	8.46 ± 0.66	10.99 ± 1.20	11.36 ± 2.13	11.13 ± 1.70	11.57 ± 2.68	13.30 ± 1.53^{A}	CA1
8	5.79 ± 0.47	7.16 ± 1.42	8.26 ± 1.94	8.13 ± 1.70	9.41 ± 2.68	11.14 ± 2.83^{A}	

ANOVA including the days 1, 2, 5, and 6 of training was significant. Values are the mean ± SEM. A, B, C, and D, show significant differences compared with days 1, 2, 3, and 4; respectively. P < 0.05.

the Pearson correlation of the peak frequency between pairs of regions across all training days was calculated, to establish whether the changes in peak frequency were similar between them, both in control conditions and after serotonin depletion in the SUMn. In the CTR group, the peak frequencies were positively and significantly correlated between the SUMn and hippocampus (both the CA1 and the DG), between the MS and the hippocampus (both the CA1 and the DG), and between the SUMn and the MS; although no significant correlation in peak frequency was observed between the CA1 and the DG (**Figure 6**). The EXP group, however, showed high positive correlations between peak frequencies of the SUMn and the hippocampus (both the CA1 and the DG), but no significant correlations were observed between the MS and the hippocampus nor between the SUMn and the MS; moreover, this group showed significant correlation in the peak frequency within the hippocampus (the DG and the CA1) (**Figure 7**). These results imply that the peak frequency of the EEG in the CTR group is related in the three structures (SUMn, MS, and hippocampus), but no relation exists within the hippocampus; in contrast, in the EXP group a closer relation occurs between the SUMn and the hippocampus with a disengagement of MS.

Coherence was compared between training days and frequency using ANOVA for repeated measures, for each group. In the CTR group no significant change was observed in the coherence between regions regarding the training days when all 6 days of training were included [$F_{(50,\ 330)} = 1.052$, $p = 0.3852$ for MS-DG; $F_{(50,\ 330)} = 1.335$, $p = 0.741$ for MS-CA1; $F_{(50,\ 330)} = 0.8018$, $p = 0.8281$ for MS-SUMn; $F_{(50,\ 330)} = 1.036$, $p = 0.4131$ for DG-CA1; $F_{(50,\ 330)} = 0.8514$, $p = 0.7519$ for DG-SUMn; and $F_{(50,\ 330)} = 1.030$, $p = 0.4236$ for CA1-SUMn]. Using the same rationale used in the RP comparisons, ANOVA tests were applied for the days 1, 2, 5, and 6 of training, and significant effects of the training days over time were thus observed for the coherence between MS-DG [$F_{(30,\ 198)} = 1.788$, $p = 0.0104$] and MS-CA1 [$F_{(30,198)} = 1.609$, $p = 0.0300$]. Paired comparisons (t-test with Bonferroni correction) showed significant increased coherence for both MS-DG MS-CA1 coherence on days 5 and 6 principally in the higher frequencies of the theta band, the coherences of MS-CA1 and MS-DG, for the

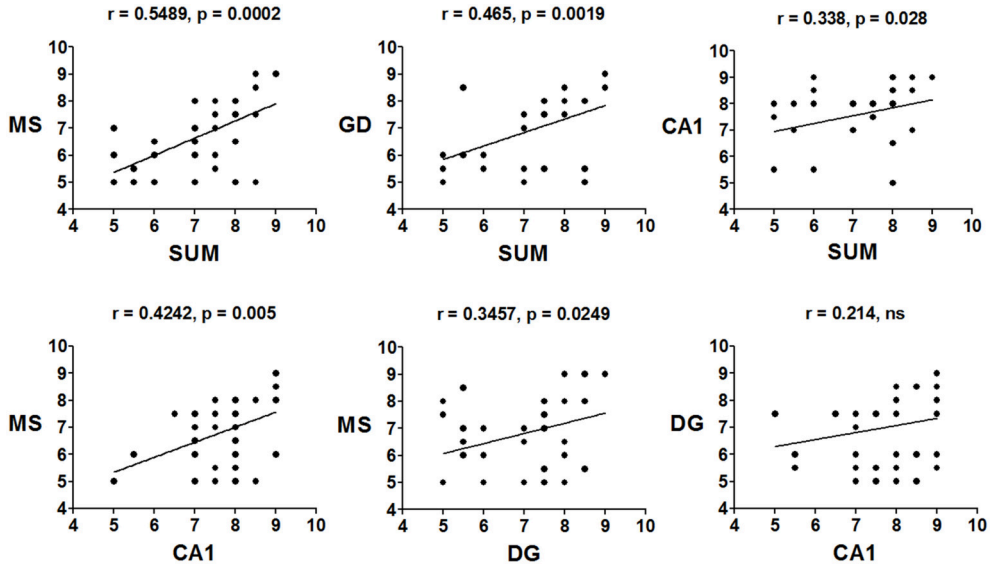

FIGURE 6 | Correlations of the mean frequency peak of the RP in the theta band (5–10 Hz) between cerebral regions, across training days, in the CTR group. Significant positive correlations between the three regions (MS, SUMn and Hippocampus), but not within the hippocampus (DG and CA1) were observed (ns, no significant).

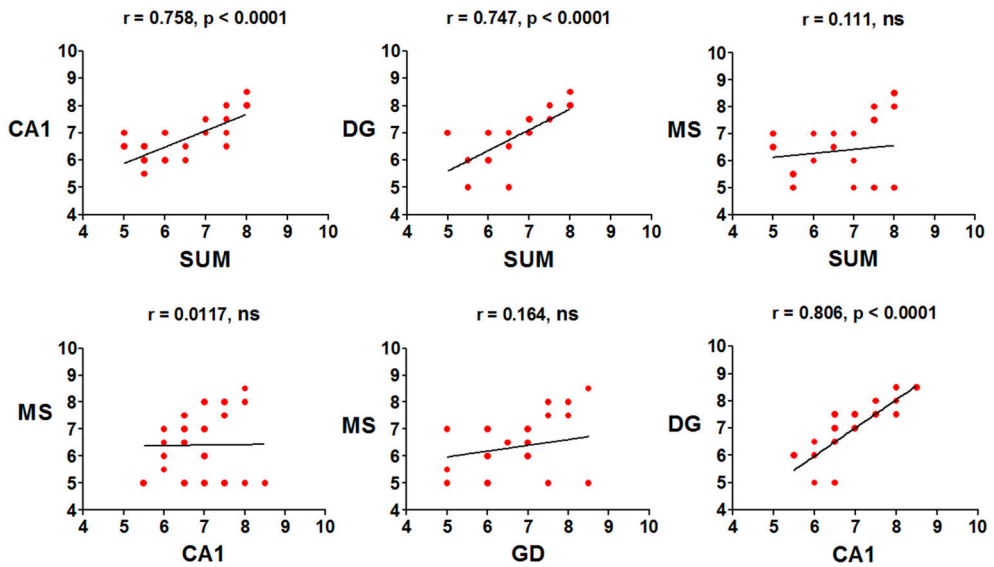

FIGURE 7 | Correlations of the mean frequency peak of the RP in the theta band (5–10 Hz) between recorded regions, across training days, in the EXP group. Significant positive correlations between the SUMn and the hippocampus and within the hippocampus (DG and CA1), were observed. No correlation between the MS and the other two regions occurred in this group (ns, no significant).

CTR group are presented in the **Figure 8**, means and significant differences are presented in the **Table 3**.

The EXP group coherences were also compared considering day and frequency using ANOVA for repeated measures. No significant effects of the training in the inter-region coherences were observed for the EXP group when all six training days were considered [$F_{(50,\ 275)} = 0.5824$, $p = 0.9887$ for MS-DG; $F_{(50,\ 275)} = 0.6685$, $p = 0.9567$ for MS CA1; $F_{(50,\ 275)} = 0.4951$, $p = 0.9983$ for MS-SUM; $F_{(50,275)} = 0.8108$, $p = 0.8130$ for DG-CA1; $F_{(50,\ 275)} = 0.5119$, $p = 0.9974$ for DG-SUM; $F_{(50,275)} = 0.4132$, $p = 0.9998$ for CA1-SUM], nor when only days 1, 2, 5, and 6 were considered. Coherences of MS-DG and MS-CA1 EEG, from the EXP group are shown in **Figure 8**.

In order to know if a shift occurred through the training days in the frequency in which the peak of coherence occurred (frequency of the coherence peak, FCP), the FCP and the

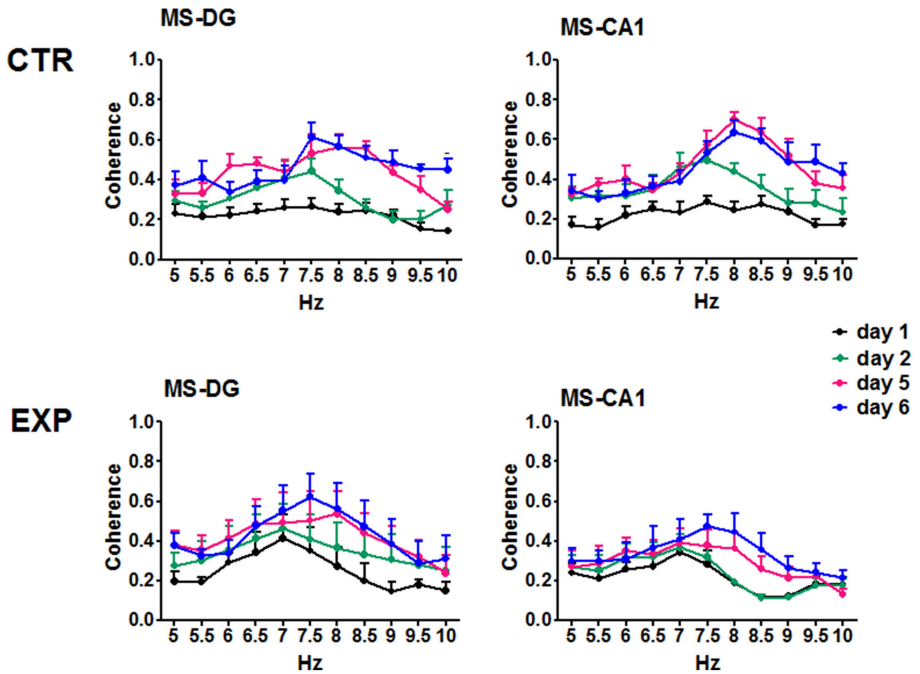

FIGURE 8 | Coherences of the EEG recorded in the MS, DG and CA1 in the two groups across training days. For the CTR group the ANOVA was significant only considering the days 1, 2, 5, and 6. Only CTR group showed increased coherence between MS-DG and MS-CA1. Mean ± SEM. Significant differences are listed in **Table 3**. $p < 0.05$.

TABLE 3 | Comparison between training days of the coherence between the EEG recorded during the searching for the platform in the Morris water maze task in the CTR group.

Hz\Day	1	2	3	4	5	6	Regions
5.5	0.214 ± 0.043	0.260 ± 0.027	0.311 ± 0.056	0.376 ± 0.070	0.331 ± 0.050^{A}	0.409 ± 0.084	
6	0.222 ± 0.037	0.306 ± 0.029	0.295 ± 0.053	0.352 ± 0.040	0.469 ± 0.059^{A}	0.338 ± 0.048	
6.5	0.241 ± 0.036	0.360 ± 0.085	0.336 ± 0.055	0.290 ± 0.035	0.480 ± 0.029^{A}	0.391 ± 0.053	
7.5	0.266 ± 0.038	0.442 ± 0.062	0.405 ± 0.074	0.438 ± 0.100	0.530 ± 0.094^{A}	0.613 ± 0.068^{A}	
8	0.238 ± 0.039	0.344 ± 0.056	0.374 ± 0.089	0.465 ± 0.082	0.561 ± 0.062^{AB}	0.565 ± 0.056^{AB}	MS-DG
8.5	0.246 ± 0.034	0.259 ± 0.043	0.368 ± 0.068	0.429 ± 0.063	0.558 ± 0.029^{AB}	0.510 ± 0.056^{AB}	
9	0.221 ± 0.027	0.198 ± 0.043	0.317 ± 0.072	0.332 ± 0.060	0.433 ± 0.035^{AB}	0.486 ± 0.060^{AB}	
9.5	0.156 ± 0.028	0.200 ± 0.043	0.228 ± 0.050	0.351 ± 0.067	0.349 ± 0.067^{A}	0.454 ± 0.020^{AB}	
10	0.144 ± 0.017	0.271 ± 0.073	0.195 ± 0.044	0.371 ± 0.058	0.251 ± 0.038	0.448 ± 0.056^{A}	
5.5	0.159 ± 0.038	0.319 ± 0.062	0.295 ± 0.084	0.349 ± 0.067	0.377 ± 0.026^{A}	0.301 ± 0.035	
7	0.235 ± 0.049	0.461 ± 0.071^{A}	0.533 ± 0.050	0.411 ± 0.084	0.432 ± 0.045	0.389 ± 0.055	
7.5	0.288 ± 0.028	0.494 ± 0.055	0.611 ± 0.045	0.529 ± 0.088	0.572 ± 0.070^{A}	0.530 ± 0.060^{A}	
8	0.246 ± 0.040	0.437 ± 0.039	0.563 ± 0.048	0.532 ± 0.075	0.700 ± 0.037^{AB}	0.635 ± 0.059^{A}	
8.5	0.275 ± 0.043	0.363 ± 0.057	0.458 ± 0.058	0.494 ± 0.076	0.634 ± 0.076^{AB}	0.594 ± 0.062^{AB}	MS-CA1
9	0.238 ± 0.045	0.280 ± 0.069	0.451 ± 0.092	0.455 ± 0.080	0.517 ± 0.085^{AB}	0.486 ± 0.096^{AB}	
9.5	0.168 ± 0.028	0.279 ± 0.065	0.328 ± 0.088	0.385 ± 0.069	0.379 ± 0.059^{A}	0.489 ± 0.083^{A}	
10	0.175 ± 0.023	0.235 ± 0.066	0.440 ± 0.071	0.375 ± 0.060	0.357 ± 0.061	0.429 ± 0.051^{A}	

ANOVA including the days 1, 2, 5 and 6 of training was significant. Values are the mean ± SEM. A and B, show significant differences compared with days 1, and 2; respectively. P < 0.05.

magnitude of the peak of coherence were compared between days of training in both groups of animals. The CTR group MS-DG, MS-CA1, and DG-SUMn FPCs, showed increases, whereas the EXP group did not show changes. In the magnitude of the peak of coherence all pairs of regions showed increases in the CTR group, whereas in the EXP group only MS-CA1, MS-SUMn, and DG-SUMn showed increase with the training. Intergroup comparison showed higher FCP in CA1-SUM and MS-CA1, and

higher magnitude of the peak for MS-CA1 for the CTR group (Figure S1). Thus, the EEG of the MS and hippocampus increased in coherence with the establishment of the memory in the CTR group but not in the EXP group.

Inter group comparisons of the coherence between pairs of regions were made using an ANOVA for repeated measures considering the factors group and frequency as independent and the training days (1–6) as repeating. MS-CA1 coherences showed significant effects both for the interaction of frequency and group [$F_{(1,\ 138)} = 17.726$, $p < 0.0001$] and for the interaction of frequency, group and day [$F_{(5,\ 690)} = 2.478$, $p = 0.031$]. Paired comparisons between frequency and group showed higher coherence between MS-CA1 regions for the CTR group from 7.5 to 10 Hz compared with the EXP group. When paired comparisons considering the training day were made, the EXP group showed lower coherences across days one to five, on day 1 in the 8.5 Hz frequency, on day 2 in the 8 and 8.5 Hz frequencies, on day 3 in the 7.5–10 Hz frequencies, on day 4 in the 9 Hz frequency and on day 5 in the 8–9 Hz frequencies. Moreover, CA1-SUMn coherences showed a significant effect of the interaction between frequency and group [$F_{(1,\ 138)} = 7.182$, $p = 0.008$], paired comparisons showed lower coherences for the EXP group in the 8.5 and 9 Hz frequencies than for the CTR group (**Figure 9**). The EXP group differed from the CTR group in both the pattern and degree of coherence.

DISCUSSION

The participation of the SUMn in place learning and memory has been controversial, with some studies reporting no or minimal effect on spatial learning and memory, after inactivation or inhibition of SUMn, and other studies implying the participation of the SUMn in retention and consolidation of spatial reference memory (Pan and McNaughton, 1997; Santin et al., 2003; Shahidi et al., 2004a; Aranda et al., 2008; Gutiérrez-Guzman et al., 2012). It was reported that lidocaine infusion into the SUMn did not affect the acquisition of an avoidance task although retention was impaired when lidocaine was infused before training. Additionally, post-training infusion caused impairments in consolidation of memory in this task (Shahidi et al., 2004b). Shahidi et al. (2004b) evaluated the effects of SUMn inactivation on spatial reference memory and spatial working memory using a Morris maze with a training schedule of 8 daily trials for 3 days. The authors did not observe alterations in reference memory when inactivation was performed before training; this implies that participation of SUMn is not crucial in the acquisition of spatial reference information. However, the author observed deficiencies when the SUMn was inactivated after the training buy prior to the probe trial. The present results showed severe impairment in spatial reference memory after SUMn serotonin depletion such that no significant reduction in the distances traveled was achieved by this group, and although the animals eventually attained a significant reduction in their escape pathways, this group searched similarly throughout the four quadrants in the probe trial. It could be interpreted that no learning was achieved by these animals based on the absence of reductions in the path lengths over the six training days; however, it was evident from the latencies in escape and the lengths of the pathways that these animals performed intermittently, presenting good performance on one trial or day and on the next trial or day and performing as badly as on the first day of training (see **Figure 2**). Thus, in spite of the severe deficiencies, the serotonin depletion did not appear to completely impair the acquisition of the spatial reference information. The deficiencies in spatial learning in the present work could be related to the impaired consolidation across days of training, according to the Shahidi results; however, some information could be acquired, although it is uncertain if the SUMn serotonin-depleted rats would have reached the control performance level with more training days. Together with the previous report in which serotonin depletion of both the SUMn and the PHn induced deficient but not absent place learning, these results support the participation of SUMn in spatial memory consolidation. Although we cannot exclude the participation of the PH, the present results show that only SUMn serotonin depletion produced deficiencies in place learning, similar to the results observed after the simultaneous serotonin depletion of the two nuclei, supporting a principal role for the SUMn in place learning.

In the present work, changes in RP were observed in CA1 theta activity, consistent with the previous studies. In addition, in the present work, evidence showed similar changes in the SUMn, that is, the decrease in RP at low frequencies (6–7 Hz) and the increase in RP at high frequencies (7.5–8.5 Hz) across training days; these changes were possibly related to the consolidation of spatial information. Furthermore, extending the brain regions previously recorded, the RP also increased in the DG (8 and 8.5 Hz), although no reduction of low frequencies was observed in the RP on this region comparing all days of training. In order to explore a possible difference in the last day compared with the first, in low frequencies, these 2 days were compared, and significant reduction was observed at 6 and 6.5 Hz frequencies the day six with respect to the first day of training. Finally, MS RP increased only in the 8 Hz frequency, this last could be an effect of broadening of the spectrum. Minor changes were evident in the EXP group only when days 1, 2 and 5, 6 were compared.

Recently a reduction in CA1 theta activity was reported in rats exposed to unexpected environmental changes, whereas they developed foraging activity (Jeewajee et al., 2008). As mentioned previously, in earlier studies assessing the relationship of theta activity with place learning ability, it was reported that the RP of the high frequency theta band (6.5–9.5 Hz) recorded in the CA1 region increased over training days in intact rats trained in the Morris spatial test, and these increases were absent in rats trained in egocentric and cue versions of the task and in aged inefficient rats (8–10). If the increased RP at high frequencies observed in the present work were due to the novelty effect for exposure of the rats to the new environment, the three groups of animals trained in the aforementioned study would have shown similar changes in their theta RP throughout the training, but only rats trained in the tasks demanding hippocampal participation presented changes in theta expression. Moreover, the changes observed in the RP in the CTR group in this study were prominent on days 5 and 6 of training, whereas changes associated with familiarity with the environment must be evident

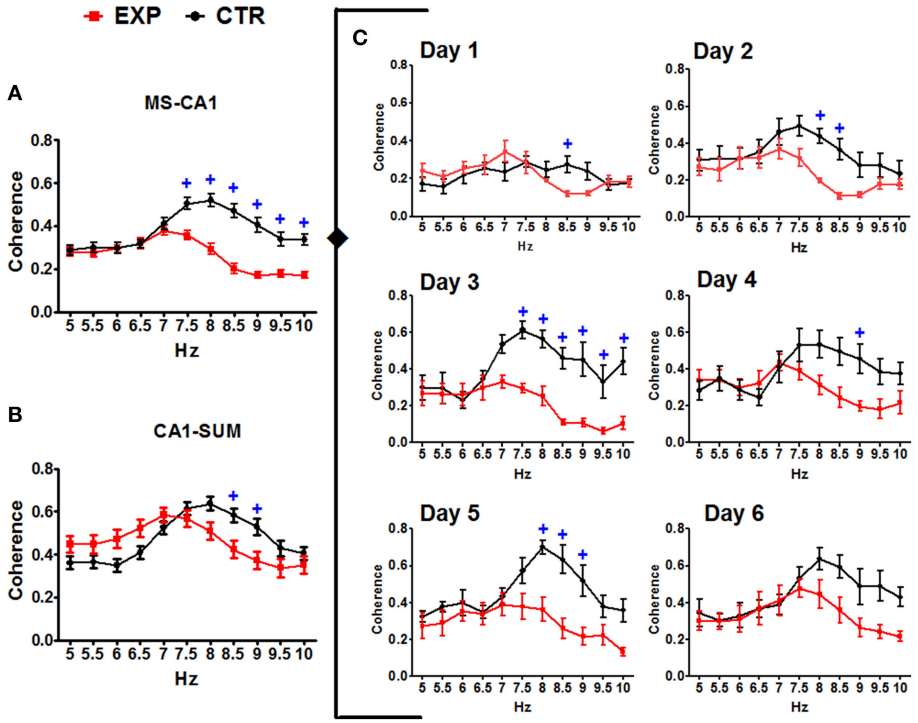

FIGURE 9 | Coherences between the EEG recorded in the MS-CA1 and CA1-SUMn, in the two groups. Inter-group comparisons in the MS-CA1 coherence (A) and in CA1-SUMn coherence (B), were observed (main effect). MS-CA1 coherence was also different through the days of training **(C)**. Mean ± SEM. +, Group CTR vs. group EXP. $p < 0.05$.

on the first days of training. Although learning could be divided into stages (essentially for the purposes of the study), learning and the consolidation of learning could occur throughout the entire training of the rats in long-term paradigms, such as the water maze. In this paradigm, there is no clear threshold indicating when the animal is learning and when it is recovering information learned in previous trials or on previous days of training; although the acquisition of more precise information continues occurring and allows the animal to develop over the days direct pathways toward the platform, it is logical to suppose that, over the training days, both consolidation of some information (spatial, motor, proprioceptive) acquired in the first trials or days could occur, whereas other information is acquired. Moreover, the recovery of the previously acquired information could occur from trial to trial or day to day of training. Thus, the different weights of the place learning processes presumably occurred at different times; it is reasonable to assume that higher acquisition of information occurred during the first 2 days, and higher consolidation and recovery of information occurred on the last 2 days, supporting the view that the processes occurred simultaneously. As in the previous work, in which depletion of SUMn/PH was realized (Gutiérrez-Guzman et al., 2012), a lack of learning-related changes in theta activity for RP was observed during processing of spatial information, not only in CA1 but also in all of the regions recorded.

The SUMn serotonin-depleted rats failed to show the increase in high-frequency theta RP related to changes over time during training, this was evident both in the peak frequency and in the RP. This failure could imply that serotonin participates in the SUMn-driven regulation of hippocampal frequency. In anesthetized rats, it was observed that neuronal SUMn theta-related firing predicted the changes in theta activity in hippocampus when sensorial stimulation occurs and also in brief episodes of theta when acceleration in frequency occur; however, the hippocampus drives the SUMn activity during spontaneous theta trains (Kocsis and Kaminski, 2006). Additionally, it has been previously observed that the ascending influence of the SUMn on hippocampal theta is not required for the occurrence theta, but it was proposed that the SUMn coding of theta frequency becomes relevant when there is a high degree of processing of information (Kirk and Mackay, 2003). Based on the absence of differences in AP through the days, we can hypothesize that changes in RP associated to learning may be caused by the same population of neurons tuning their synaptic oscillations within the range of the theta band, from lower to higher frequencies, effect that was absent in the EXP group. Thus, an increase in RP of one hertz (e.g., 8 Hz) could occur when, in fact, their power increased or when the power of all of the other frequencies decreased, or the two phenomena occurred simultaneously whatever the mechanism, it implies the predominance of high frequency activity.

The changes in RP coherence in CTR rats could reflect increased communication between the MS and hippocampus possibly related to consolidation of spatial information and

recovery of the same. In accordance, it was reported that hippocampus weakly conduced SUMn activity during the initial training, in a 1-day test of spatial learning (16 trials), whereas during the last trials of training the direction of the influence inverted so that the SUMn directed the hippocampal activity, which was also associated with an increase in coherence between the two regions during the last training trials, when the consolidation of information takes place (Ruan et al., 2011). Differences in the training paradigm could account for this because in the present work, 4 daily trials were given to the rats and more gradual process of consolidation could be occurring in comparison with the collapsed training (16 trials) in 1 day. However, in the present work, we did not observe increased coherence between the SUMn and hippocampus across training days in control rats, but the frequency of the peak of coherence for DG-SUMn increased with the days, to be significant on day 6; moreover increased coherence was evident between the MS and the hippocampus (DG and CA1) on days in which consolidation occurred more preferentially (days 3–6); and increase in the frequency of the coherency peak occurred for MS-DG (gradual but significant on day 6).

Thus, the learning of the spatial task was accompanied by changes in power in all regions recorded and increased coherence between MS and the hippocampus across training days in CTR animals; these changes were absent in the EXP group. Surprisingly, MS theta activity did not show changes in relation to the SUMn in coherence, and the RP increased only in the 8 Hz frequency across training days, this was unexpected in view of the modulator role of the SUMn on MS activity. The absence of increases in the RP of high-frequency theta in the SUMn serotonin-depleted rats as well as the absence of increases in coherence between the MS and the hippocampus could underlie the inefficient performance of these animals. In support of this idea, the peak frequency showed in each region was highly correlated in CTR animals (even though scant direct connection has been reported between the SUMn and the CA1) (Haglund et al., 1984), whereas in the EXP group the RP peak frequency between MS and the other two regions was unrelated, and there were highly correlated peak frequencies within the hippocampus (DG with CA1) and between hippocampus and the SUMn. This result, together with the minor coherence between the MS and the CA1 and the MS and the DG in SUMn serotonin-depleted animals (compared with the CTR group) would imply a reduced communication between the MS and the hippocampus caused by the withdrawal of the SUMn serotonin influence. The influence of SUMn could be necessary to entrain the information flux in the MS-hippocampus circuit, during consolidation of memory, and the absence of serotonin appears to alter the fine-tuning of the SUMn activity required for this purpose. Instead, the EXP animals appear to be entrained in a closed circuit between the hippocampus and SUMn, and this would impair the consolidation of memory (**Figure 10**).

The SUMn receives projections from medial and lateral mammillary (MM) nuclei (Gonzalo-Ruiz et al., 1992), and the MM theta-related rhythmic firing originates from a descendent influence from the hippocampus, whereas SUMn theta influences ascend through the input received from the RPOn and TPPn (Kocsis and Vertes, 1994; Kirk et al., 1996; Kirk, 1997). Although in the present work we cannot rule out the possibility of some leakage of 5,7-DHT to the adjacent MM region, the descendent origin of MM theta supports the idea that the changes observed in the EXP group are mainly due to SUMn serotonin depletion. Moreover, the role of the MM in place learning has been evaluated, and no changes in a spatial reference memory similar to those reported in the present work were observed, although, mild to severe deficiencies occurred after total or partial MM lesions when spatial working memory was implicated, e.g., in T maze delayed tasks and in Morris maze and radial arm maze working memory tasks (Santin et al., 1999; Vann and Aggleton, 2003; Vann, 2005). A previous study reporting place reference memory deficits after MM lesions included animals that suffered bilateral destruction of the SUMn in addition of the MM damage (Sutherland and Rodriguez, 1989); moreover, increased cFos expression occurred in the medial MM nucleus after a working memory task but not after a spatial reference memory task (Santin et al., 2003). Thus, it is unlikely that the deficiencies observed in spatial reference memory in Morris maze in the present work were due to serotonin depletion that extended into the MM.

The SUMn is monosynaptically connected to the DG in a segregated pattern, the lateral SUMn synapses with the dorsal DG and the medial SUMn synapses with the ventral DG (Ohara et al., 2013), where it makes glutamatergic and GABAergic/Glutamatergic contacts both on granule cells and on GABAergic neurons (Nitsch and Leranth, 1996). The SUMn also sends glutamatergic afferents to the CA2/CA3 regions of the hippocampus (Soussi et al., 2010), and is also reciprocally connected to the MS (Vertes, 1988, 1992), through a glutamatergic input onto cholinergic and GABAergic MS neurons and a GABAergic descending input from the lateral septum (LS) onto the lateral SUMn (Leranth and Kiss, 1996). Unlike the abundance of knowledge about the connectivity of the SUMn, there is scant information about the serotonergic projections to and receptors, through which serotonin influences the neuronal activity, on the SUMn. A moderate concentration of serotonergic terminals was reported to project to the lateral SUMn and slightly denser concentration was reported in the medial SUMn (Moore et al., 1978; Vertes and Martin, 1988; Vertes et al., 1999). In addition, the presence of $5HT_{1C}$ and $5\text{-}HT_2$ receptors, particularly the 5-HT2A receptor with a moderate density both on the soma and on dendrites of neurons, has been reported on the SUMn (Wright et al., 1995; Cornea-Hébert et al., 1999). Whereas the effect of SUMn manipulations on CA2/CA3 is relatively unexplored, CA1 pyramidal excitability is suppressed and theta activity activated by SUMn carbacol microinjections (Jiang and Khanna, 2006) and SUMn stimulation increases the population of spikes in the DG evoked by stimulation of the perforant pathway in anesthetized rats (Mizumori et al., 1989). Additionally, the SUMn is known to modulate septal cell firing and hippocampal theta frequency in anesthetized rats, in which procaine injection into SUMn produced the attenuation of both frequency and amplitude of hippocampal theta (Kirk and McNaughton, 1993);

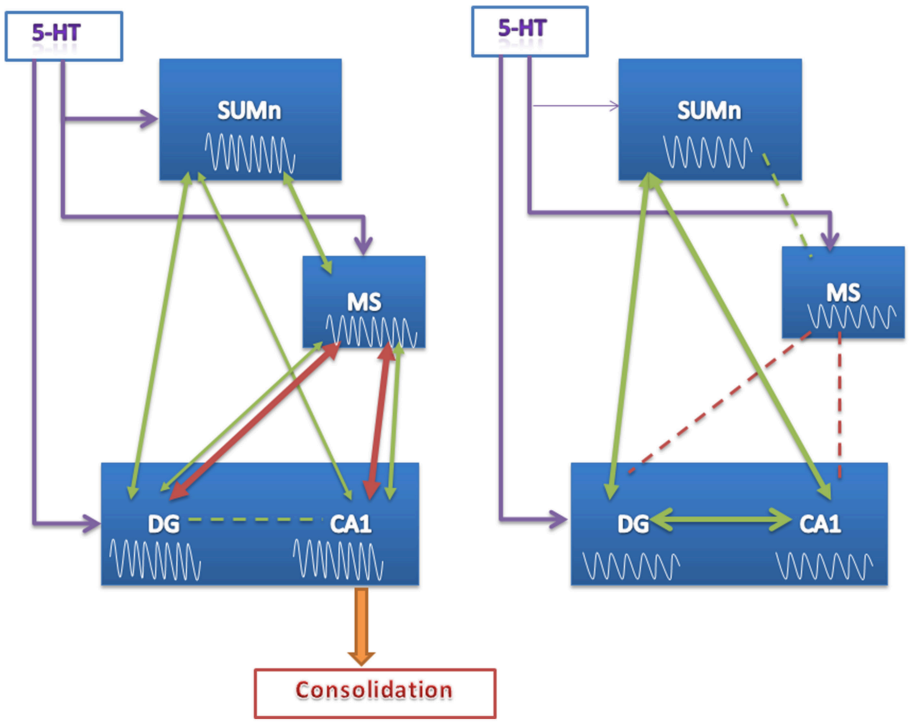

FIGURE 10 | Proposed model of function of the SUMn, the MS and the hippocampus circuit during learning after SUMn serotonin-depletion. The model represents the results observed principally the last days of training. EEG RP values are represented into the boxes, showing that no increase in high frequency RP was observed in the EXP group. Green arrows represent values of frequency peak; red arrows represent values of coherence. The thickness of the arrows represent the grade of coherence or correlation; broken lines represent the lack of coherence or correlation. When 5-HT was reduced in the SUMn, the circuit appear to entrain into a closed mode between SUMn and hippocampus. The lack of communication between MS and CA1 would impair the consolidation of memory.

however, it was observed that the electrolytic lesioning of the SUMn did not affect the movement-related theta frequency in behaving rats (Thinschmidt et al., 1995). In this manner, it is highly speculative attempt to explain what could be the consequence of reduced serotonin on the electrical activity at the neuronal level in the SUMn and the repercussion on the MS. Although it is possible support that the tuning of theta during movement-related information processing (e.g., place learning) and not the movement-related theta could be disrupted in SUMn serotonin-depleted rats, this remains speculative. To our knowledge, no other evidence of SUMn modulation of theta activity during learning in awake rats exists; however, whatever the effect, the present results support the role of the serotonin acting on the SUMn, in the modulation of the hippocampal theta activity underlying the processing of spatial information and in the consolidation of this information.

In conclusion, reduction of serotonin in the SUMn produced deficiencies in place learning ability and altered pattern of hippocampal, septal, and SUMn theta learning-related activity, in the rat.

AUTHOR CONTRIBUTIONS

All authors participated in the experimental design, experimental work and data analysis. In addition, JH and MO participated in the redaction of the final article and all four authors discussed the contents and interpretations of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphar.2015.00250

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HIPPOCAMPAL STRATA THETA OSCILLATIONS CHANGE THEIR FREQUENCY AND COUPLING DURING SPATIAL LEARNING

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Abstract—The theta rhythm is necessary for hippocampal-dependent spatial learning. It has been proposed that each hippocampal stratum can generate a current theta dipole. Therefore, considering that each hippocampal circuit (CA1, CA3, and Dentate Gyrus (DG)) contributes differently to distinct aspects of a spatial memory, the theta oscillations on each stratum and their couplings may exhibit oscillatory dynamics associated with different stages of learning. To test this hypothesis, the theta oscillations from five hippocampal strata were recorded in the rat during different stages of learning in a Morris maze. The peak power, the relative power (RP) and the coherence between hippocampal strata were analyzed. The early acquisition stage of the Morris task was characterized by the predominance of slow frequency theta activity and high coupling between specific hippocampal strata at slow frequencies. However, on the last training day, the theta oscillations were faster in all hippocampal strata, with tighter coupling at fast frequencies between the CA3 pyramidal stratum and other strata. Our results suggest that modifications to the theta frequency and its coupling can be a means by which the hippocampus differentially operates during acquisition and retrieval states.

Key words: Morris water maze, electrode array, learning and memory, theta oscillations, navigational learning.

INTRODUCTION

The theta rhythm is a predominant pattern in hippocampal circuits during REM sleep and during active behaviors that involve external-world information processing, such as walking, running, jumping, swimming, rearing, head moving, sniffing, and whisking (Vanderwolf, 1969; Winson, 1978; Leung, 1984; Vinogradova, 1995); during periods of immobility with highly aroused states due to a previously conditioned stimuli (Vanderwolf, 1969; Whishaw, 1972; Bland, 1986) or the presence of a predator (Sainsbury et al., 1987); and during time discrimination periods (Nakazono et al., 2015). This activity pattern is present and necessary for hippocampal-dependent spatial learning (Winson, 1978; Mitchell et al., 1982; Mizumori et al., 1990; Turnbull et al., 1994; Buzsáki, 2002; McNaughton et al., 2006b; Shirvalkar et al., 2010). However, the mechanism by which theta activity contributes to hippocampal function is unknown.

It has been suggested that the theta rhythm may synchronize neural populations to enable information transfer within and across neural circuits (Sirota et al., 2008; Gordon, 2011; Buzsáki and Watson, 2012). Consistent with this idea, several studies have shown that the gamma oscillations and firing of neurons in the hippocampus (Bragin et al., 1995; Csicsvari et al., 2003; Colgin et al., 2009; Montgomery et al., 2009) and neocortical areas are phase-locked to the hippocampal theta rhythm (Jones and Wilson, 2005; Siapas et al., 2005; Canolty et al., 2006; Sirota et al., 2008; Colgin et al., 2009). Additionally, it has been found that the oscillatory activity in other circuits, such as the prefrontal cortex, amygdala, striatum, medial septum, and supramammillary nucleus, can be synchronized with hippocampal theta oscillations during cognitive processes (Seidenbecher et al., 2003; DeCoteau et al., 2007; Adhikari et al., 2010; Benchenane et al., 2010; Hernández-Pérez et al., 2015). Therefore, the theta rhythm may participate in the local computational operations in the hippocampal circuits and in the synchronization of widely distributed subcortical and cortical networks in order to construct temporary functional circuits. Moreover, the theta rhythm cannot be considered as a global clock, because the global theta activity recorded in LFP depends on the proportional contribution of multiple theta dipoles found in the different hippocampal strata (Feenstra and Holsheimer, 1979; Buzsáki et al., 1983; Leung, 1984; Montgomery et al., 2009). Additionally, the generation of traveling waves that propagate from the septal to the temporal axis has been

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Abbreviations: ANOVA, analysis of variance; DG, Dentate Gyrus; LFP, local field potential; RP, relative power; SAS, synchronizing ascending system; sm, moleculare stratum; sml, lacunosum-moleculare stratum; spCA1, pyramidal stratum (CA1); spCA3, pyramidal stratum (CA3); sr, radiatum stratum.

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demonstrated (Petsche and Stumpf, 1960; Lubenov and Siapas, 2009; Patel et al., 2012).

The intra-hippocampal circuit has a highly organized connectivity. In this sense, the CA1 area integrates information coming from CA3 and entorhinal cortex areas, whose afferents arrive in the radiatum and lacunosum-molecular strata, respectively (Witter and Amaral, 2004). A characteristic of hippocampal circuitry is that each excitatory input is matched by a specific inhibitory input provided by GABAergic interneurons (Freund and Buzsáki, 1996; Witter and Amaral, 2004; Klausberger and Somogyi, 2008). It is also known that a wide subpopulation of interneurons is phase-locked to the theta rhythm (Somogyi and Klausberger, 2005); therefore, it has been suggested that the interplay of excitatory and inhibitory theta patterns may generate a current dipole at each layer or stratum (Buzsáki, 2002; Montgomery et al., 2009). In this picture, the theta oscillations reflect the activity in the circuits as well as the interaction among themselves. Therefore, the theta oscillations may change as a result of distinct computational operations in the intra-hippocampal circuits during the mnemonic process.

An interesting recent study found stratum-specific variations in theta oscillations during the performance of a memory task (Montgomery et al., 2009); however, that study, like a large amount of other work, focused primarily on the study of the dynamics of hippocampal theta during stage retrieval and/or decision making (Schmidt et al., 2013; Belchior et al., 2014), analyzing the oscillatory patterns during the performance of a memory task that has already been learned. Instead of this process, we are interested in studying the oscillatory dynamics of the theta frequency of intra-hippocampal circuits during navigational learning in order to understand how the oscillators of each stratum are organized during the learning stage. It is proposed that if the theta activity in the hippocampal strata plays a role in the processing of spatial information, it may vary in function of learning from the early to late training phase.

For this purpose, we used the place version of the Morris water maze task, which is a successful test to evaluate hippocampal-dependent navigational learning. In previous work, modifications in hippocampal theta oscillations during the learning of the water maze in the CA1 and Dentate Gyrus (DG) have been shown (Olvera-Cortés et al., 2002, 2004; Gutiérrez-Guzmán et al., 2011, 2012; Hernández-Pérez et al., 2015), but the dynamics of the intra-hippocampal circuits during spatial learning of the Morris maze have not been addressed. Therefore, in the present study, we recorded theta oscillations concurrently in multiple hippocampal strata using an 8-electrode (180-micron spacing) linear array in order to record the oscillations generated in the hippocampal strata: Pyramidal CA1 (spCA1), Radiatum (sr), Lacunosum-Moleculare (sml), Moleculare (sm) and Pyramidal CA3 (spCA3). Because it is known that the CA1, CA3 and DG hippocampal areas differently contribute to distinct aspects of spatial learning and memory (Marr, 1971; Treves and Rolls, 1992; Kesner et al., 2004; Lee and Kesner, 2004; Colgin et al., 2008; Yassa and Stark, 2011; Carr and Frank, 2012), our main objective was to analyze whether stratum-specific modifications in the frequency and/or coherence theta occur that reflect specific oscillatory dynamics associated with different stages of navigational learning.

EXPERIMENTAL PROCEDURES

Subjects

Six Sprague–Dawley male rats (age, 4–5 months; 450–500 g) were used. All experiments were performed in accordance with the National Institute of Health guide for the care and use of laboratory animals (NIH Publications No. 80-23) and with the "Norma Oficial Mexicana para el uso de animales de laboratorio" (NOM-062-ZOO-1999). This work was approved by the Research and Ethics Committee of the Instituto Mexicano del Seguro Social, México. The rats were maintained under standard vivarium conditions with 12 h/12 h light–dark cycles, with the temperature controlled at 22 ± 2 °C, and with food and water available ad libitum. All experiments were carried out during the light period, starting at 10:00 am. The experiment test was carried out in groups of three animals. The order in which to start the behavioral test was counterbalanced in such way that each animal started the training between 10:00 and 11:00 am.

Electrode construction

To record the theta oscillations corresponding to each hippocampal stratum, an electrode array was built with eight nichrome wires (25 μm in diameter) and was mounted inside a guide cannula. The electrodes were aligned and spaced 180 μm apart in order to reach five hippocampal strata: spCA1, sr, sml, sm, and spCA3 (Fig. 1A, B). The electrode array was attached to Triangle Biosystems International© Electrode Interface Boards (EIB), and a Microdrive was built (Vandecasteele et al., 2012) in order to adjust the electrode array in an optimal recording position.

Surgical procedures for the implantation of electrodes

Each rat was anesthetized with a mixed solution of ketamine (60 mg/kg) and xylazine (10 mg/kg) and was fixed in a stereotaxic instrument (AnyAngle, Stoelting). The scalp was disinfected with iodine solution, and local anesthetic (Lidocaine, 0.5 ml of 20 mg/ml) was infiltrated subcutaneously before any surgical incision. The skin was gently retracted from the skull, and on the surface of the exposed skull, craniotomy (1.8 mm in diameter, Trephine bone drill bits, BASi) was performed over the right dorsal hippocampus to implant the electrode array (HPC, 3.8 mm posterior, and 3.5 mm lateral to bregma). For two rats, the electrode array was slowly lowered to 3.0 mm below the brain surface and fixed to the skull with dental acrylic, while for the four remaining rats, the electrode array was slowly lowered to approximately 1.8 mm below the brain surface and carefully fixed to the Microdrive with dental cement. The Microdrive was

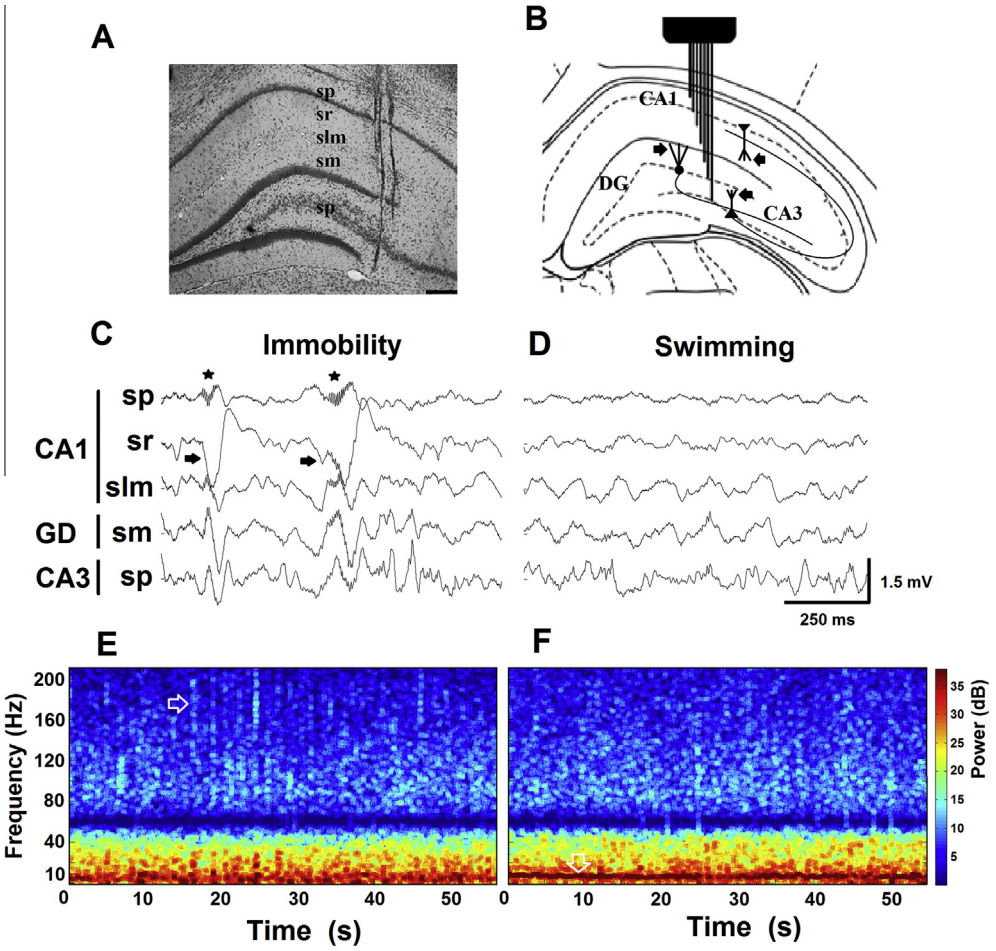

Fig. 1. Position of the recording electrodes and electrical activity profile of the hippocampal strata. (A) Photomicrograph showing tracts corresponding to some electrodes from an electrode array of eight recording sites, schematized in figure (B). The organization of internal and external connections (arrows) are represented. (C) and (D) Profile of stratum-specific activity; the sharp-wave/ripple (SPW-R) events during immobility and the theta oscillations during swimming behaviors are shown. The ripple waves (stars) are present in the pyramidal stratum, and the sharp waves are in the radiatum stratum (arrows). (E) and (F) The spectrogram from the pyramidal stratum during immobility and swimming.

attached to the skull with dental cement. To secure the implanted array, a plastic base was placed to cover the implant. A screw was inserted above the cerebellum to be used as a reference and ground site. After the surgery, the rats were maintained in a recovery room under standard care for 14 days before starting the training in the water maze.

Recording procedures

For the four rats implanted with a movable electrode array, the electrophysiological signals were pre-amplified 100X with a Triangle BioSystems headstage of 16 channels. For the two rats with a fixed electrode, the signal was amplified 1X with a Neurotek-IT headstage. To avoid a short-circuit from possible contact with water during the behavioral task, the headstage and connectors were protected with a plastic tube that was attached to the cemented base located in the animal connector. All connections on the head of the rats were completely wrapped with parafilm in order to prevent them from wetting during swimming. The signal was sent through a motorized commutator device with 32 channels (Neurotek-IT) to an EEG recording system, where it was amplified 50× in rats whose signal was pre-amplified 100× and 1000× in rats whose signal was pre-amplified 1×. The signal was filtered (1–6000 Hz) with a model 15A54 Grass amplifier. A notch filter of 60 Hz was used, and the signal was digitized at 25 kHz with a DataWave Technologies data acquisition system. The local field potential (LFP) was down-sampled to 1.2 kHz. The behavior of the animals was tracked via a small light-emitting diode attached to the headstage, sampled at a rate of 30 Hz, and was analyzed with DataWave software (VideoBench).

Microarray adjustment and estimation of electrode array position

For the four rats implanted with a movable electrode array, a week after the surgery, the electrode array was lowered in steps of 50 μm, until the desired hippocampal strata were reached. The electrode position for each hippocampal stratum in the six rats was estimated through electrophysiological parameters (Bragin et al., 1995; Montgomery and Buzsáki, 2007; Montgomery

et al., 2009). Spontaneous activity, such as ripple oscillations and multiunit activity, was used to identify the pyramidal CA1 stratum, and the radiatum stratum (sr) was defined by sharp waves (Fig. 1C, E) and a phase shift of approximately 90° with respect to the pyramidal layer theta activity at CA1. The lacunosum-moleculare stratum was characterized by the most robust theta activity and theta phase reversal at 180° with respect to CA1 pyramidal layer theta activity, while the molecular layer presented a theta phase reversal with respect to CA1 pyramidal layer theta activity, similar to the lacunosum-moleculare stratum, but with prominent gamma activity (Fig. 1D). Finally, the CA3 pyramidal stratum was characterized by multiunit activity with burst firing during ripple oscillations and prominent gamma oscillations (Fig. 1C, D). For the rats implanted with a movable electrode array, the experimental tests were conducted 2 days after the electrode array was placed in its final position. At the end of the experiments, the rats were deeply anesthetized (sodium pentobarbital 35 mg/kg) and intracardially perfused with buffered formaldehyde solution (4%). Then, the brains were removed from the skull, and the electrodes' positions were histologically verified (cresyl violet staining) (Fig. 1A).

Behavior and EEG recording

The behavioral test of the Morris water maze was conducted in a swimming pool (1.5 m in diameter, filled with water dyed blue by the addition of gentian violet) placed in a room with extra-maze visual cues. The water temperature was maintained at 23–24 °C. The goal was a circular platform of 9 cm in diameter submerged 2 cm below the surface of the water. The ratio of pool surface area to platform area, calculated as the area of pool/area of platform in cm^2, was 280, ensuring optimal task difficulty to evaluate hippocampal function on spatial learning (Vorhees and Williams, 2006).

The water maze test was carried out over four days, with six trials per day. At each trial, the rats were placed facing the tank wall and randomly released from four conceptually divided quadrants to search for the goal for 60 s, and if the rat failed to reach the platform, it was guided to the goal. The rat remained for 15 s on the platform, and it was subsequently moved to a maintenance box for 120 s (inter-trial interval) until the next trial. To avoid sudden jerks of the animal head during the inter-trial period, the animal was carefully dried with a towel after each trial in the water maze. This procedure can also reduce the probable cooling associated with the training, since it has been reported that a reduction in core temperature (Whishaw and Vanderwolf, 1971) and long periods of swimming in cool water (22 c) before starting the training in the Morris maze modifies the theta frequency (Pan and McNaughton, 1997). The swimming path length and the swimming speed of the training tests were calculated using Video-Bench software.

Furthermore, to concentrate different stages of task performance (from early acquisition to successful learning) on distinct days of training, a protocol of six daily trials for four days was established. In addition to the fact that encoding and retrieval processes cannot be separated in the Morris maze task, it is supposed that there were days in which the information processing was putatively different, where the balance between different processes may change across training. The acquisition of information to solve the task may dominate in early training (day one), while the recovery of memory may predominate in later training (day four), when the best performance is achieved. Additionally, in order to discard unspecific changes in frequency and coherence theta associated with training days, we compared the theta activity under two conditions (baseline and spatial learning) across the training days. The baseline condition corresponds to the walking periods in the maintained box before starting the learning task, while the spatial learning condition corresponds to the Morris water maze performance task (during the swimming stage).

In addition, to test whether our training protocol produces changes in body temperature, a second group (five animals) was placed in the water maze to spend the same time swimming in the water under the same water temperature as the EEG recorded group. The rectal temperature in the five animals after each trial in the water maze was measured.

Data analysis

The data were imported into MATLAB (Mathworks, Inc.) to visually eliminate artifacts using the EEGLAB software program (Delorme and Makeig, 2004). LFPs were analyzed using custom programs adapted from Ken's MATLAB library, written by Ken Harris and available at: http://osiris.rutgers.edu/Buzsaki/software. The power of theta oscillations was estimated through power spectral density analysis using Welch's periodogram method. The coherence was analyzed using the Matlab function 'cohere' (Signal Processing Toolbox). Coherence estimates were computed as magnitude-squared coherence Cxy(f) using Welch's averaged, modified periodogram method and the following formula:

$$C_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)}$$

where $P_{xx}(f)$ and $P_{yy}(f)$ are the power spectral densities of each individual signal $x(t)$ and $y(t)$, and $P_{xy}(f)$ is their cross PSD. Due to the coherence function being a measure that reflects a linear association between two signals, it might be considered a measure that provides information about functional interactions between brain regions (Sabolek et al., 2009).

The spectral analysis was conducted in 2-s windows to ensure a theta frequency resolution of 0.5 Hz. The mean of the theta activity power and coherence values on each day was obtained from the six daily trials. The absolute power of the theta activity calculated in bins of 0.5 Hz was converted in relative power (RP) with respect to total power (100%) of the band (4–12 Hz) with the objective of analyzing the relative contribution of each 0.5 Hz to the theta band. Comparisons were made of the RP within the range of 4–12 Hz in each

stratum between days and frequency. Using an ANOVA for repeated measures and a paired t-test with a Bonferroni correction, the normality D'Agostino and Pearson omnibus tests were also conducted. The primary interest in this study was to analyze variations in frequency and coherence in the theta band associated with the processing of spatial information in order to investigate whether distinct theta band frequencies play a role in navigational learning.

RESULTS

Behavioral water maze

The learning was assessed as a reduction in the distance measure. The comparison of swimming path lengths to find the platform (distance) (repeated measures ANOVA, training day factor and Tukey's test) showed changes ($F3,19 = 13.67$, $p = 0.0004$), as the animals reduced the length on days two to four compared to day one ($p < 0.05$) (Fig. 2A). The swimming velocity did not show changes over the course of the training days ($F3,19 = 0.8751$, $p = 0.4811$) (Fig. 2B) or across the training trials of day one ($F5,29 = 0.4328$, $p = 0.8203$) (Fig. 2C) (repeated measures ANOVA). The statistical analysis of path length and swimming velocity was calculated from the same five animals used for the power spectral analysis.

A progressive increase in peak frequency occurred during spatial learning but not in the baseline condition

Starting from the idea that the theta oscillations may be the way through which the intra-hippocampal networks organize the information (cognitive content) that is processed in different stages of spatial learning (encoding, consolidation and retrieval), the variations in frequency and/or coupling among theta oscillations generated in the different intra-hippocampal circuits may support the cognitive operations that underlie spatial learning.

To assess this hypothesis, two types of theta oscillations were analyzed and compared. First, were those oscillations evoked during motor activity that involves behaviorally relevant cognitive content. These correspond to the oscillations registered in the four training days during the performance of the Morris water maze task (spatial learning condition). The second were the theta oscillations recorded during motor activity that do not require cognitive effort, such as spontaneous walking episodes in the maintenance box before the start of the water maze test on the four training days (baseline condition). The representative traces of LFP (local field potential) of the stratum radiatum of one animal in spatial learning and baseline conditions are shown in Fig. 3A. The power spectral analysis was conducted using the EEGs obtained from five rats, except for the spCA1, where the data came from four rats. The representative spectrogram from one animal in spatial learning conditions is shown in Fig. 3B.

For the spatial learning condition, the peak frequency on each day was calculated by averaging the peak frequency of the six daily trials, while for the baseline condition, the peak frequency was calculated from 90 s of walking episodes on each day. To assess the variation in the peak frequency across the training days in the two conditions, a two-way ANOVA for repeated measures, with factors of training days (one to four) × condition (spatial learning, baseline), was conducted. The interaction for the two factors was significant at all strata: spCA1 ($F3,18 = 7.997$, $p = 0.0013$), sr ($F3,24 = 7.528$, $p = 0.001$), slm ($F3,24 = 18.96$, $p = 0.0001$), sm ($F3,24 = 13.59$, $p = 0.0001$) and spCA3 ($F3,24 = 6.162$, $p = 0.002$) (Fig. 4A). The post hoc analysis revealed changes for the spatial learning condition, where the peak frequency became progressively faster across training days in the spCA1 ($p < 0.05$), sr ($p < 0.05$), sml ($p < 0.05$), sm ($p < 0.05$), and spCA3 ($p < 0.05$) (Fig. 4A). However, for the baseline condition, the peak frequency was unchanged (≈7.8–8 Hz) across the training days in the strata, spCA1, sr, sm and spCA3, but a reduction in peak frequency was observed on day four with respect to day two in the sml stratum ($p < 0.05$) (Fig. 4A). These results show that the theta oscillations expressed during spatial learning were different from theta oscillations evoked for motor activity during the baseline condition. Highlighting the relationship between peak frequency and spatial learning stages, a slow frequency theta (6.5 Hz) predominated in the early acquisition stage of learning (mainly on the first day), whereas a fast frequency (8 Hz) was present at the final stage of learning (predominantly on the last training day).

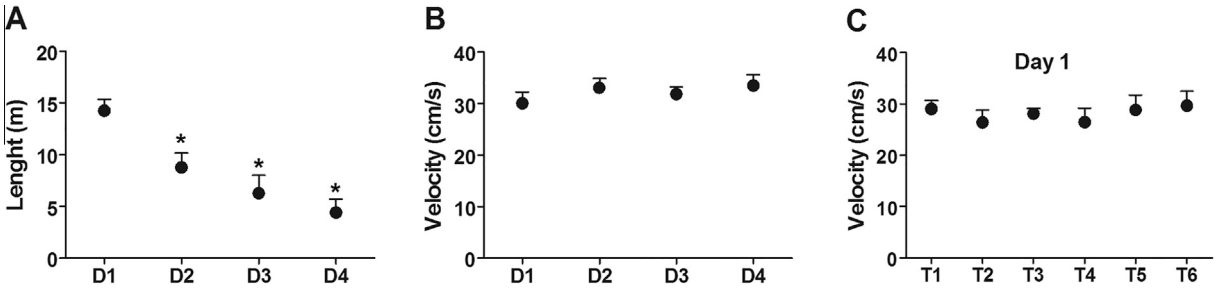

Fig. 2. Spatial learning is evident as a reduction in path length (A) (means ± S.E.M.). The swimming velocity (means ± S.E.M.) was unchanged throughout the learning test days (B) and the trials on day one (C). In section B, the values represent the average of six trials per day. D = Day, T = trial. $^{*}p < 0.05$.

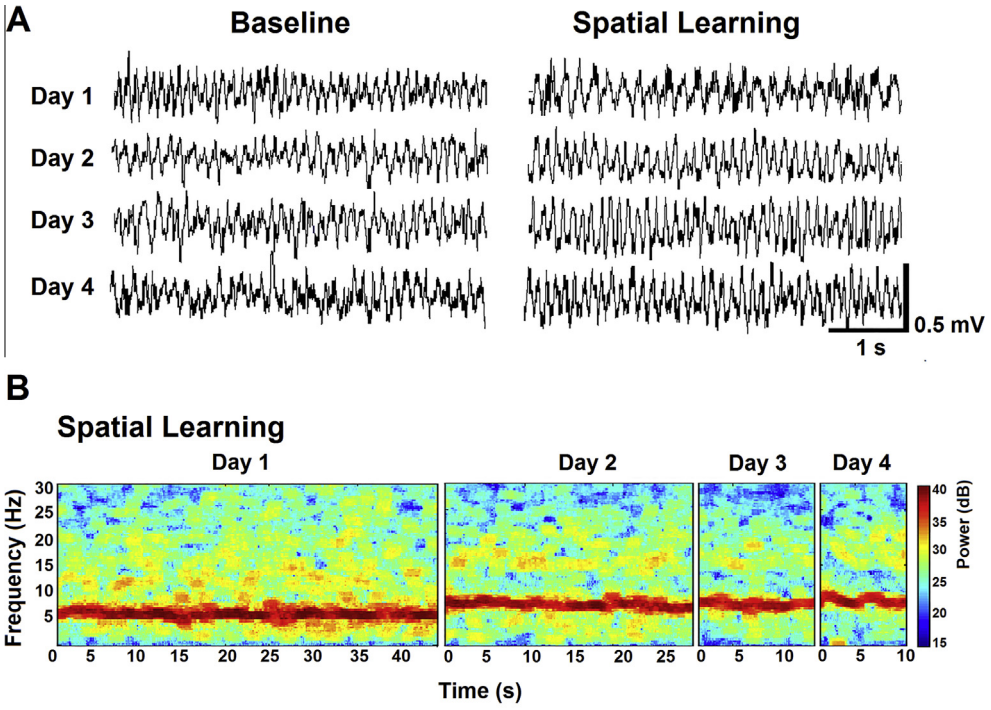

Fig. 3. (A) Representative traces of oscillatory activity recorded in the radiatum layer from an animal during baseline theta activity (walking in maintenance box) and spatial learning (swimming during the task) throughout the four days of the task. (B) Representative spectrogram of one animal during the Morris maze performance throughout the four training days.

Changes in peak frequency during spatial learning are not correlated with swimming velocity

To reveal whether a relationship exists between theta frequency (during spatial learning) and swimming velocity, a Pearson correlation (peak frequency and swimming velocity) was calculated for the data obtained in the sr, but the analysis did not show any significant correlation for the four training days (Fig. 4B). Additionally, because the relationship between frequency and swimming velocity may be obscured when analyzing the frequency and velocity average of a whole trial, epochs of two seconds were analyzed in blocks of two trials on day one as well as in a block of six trials on day four (Fig. 4C). A significant negative correlation ($r = -0.3076$, $p = 0.0007$) was found in the trials (5–6) of day one, indicating that slow frequencies were present during high swimming velocity. On the other hand, in order to determine whether the different swimming velocities modify the theta frequency, the mean frequency was calculated to different velocities in 5 cm/s bins (one-way ANOVA, factor swimming velocity and Tukey's test). An association between different intensities of swimming velocity and theta frequency was not found (Fig. 4D). Only the third block (5–6 trials) of day one ($F6,117 = 2.714$, $p = 0.0169$) showed a higher frequency for the 25 cm/s velocity over the frequency for the 45 cm/s velocity ($p < 0.05$). In addition, a progressive diminution in frequency across blocks of training trials (even over all intensities of swimming velocities) on day one was observed (Fig. 4D). These results show that the changes in theta activity during the spatial learning task cannot be explained by variations in swimming velocity.

Additionally, the relationship between swimming path length and peak frequency was assessed with a Pearson correlation, and the results showed a significant negative correlation ($r = 0.494$, $p = 0.006$) among path length and peak frequency on day one, while on the rest of the days of the task, there were no correlations between the two variables (Fig. 4B). The relationship found on day one (the early acquisition) may suggest that when the animals acquire behaviorally relevant novel information (to solve the learning task), the theta peak frequency is slower.

On the other hand, it is known that changes in core temperature modify the theta frequency in rats. However, the training protocol did not robustly change the core temperature in an additional animal group that was placed in the water maze to spend the same time under the same water temperature as the previous group. The core temperature (one-way ANOVA, Tukey's test) shows changes over the course of the training days ($F3,19 = 3.556$, $p = 0.0001$). Days 1 and 2 were characterized by a minimum reduction of 0.9 and 0.5 °C, respectively, in comparison with its initial temperature (37.8 °C) ($p < 0.05$) (Data not shown). Therefore, it seems that changes in the core temperature were not strong enough to produce modifications in theta frequency across training days; however, any effect of temperature changes cannot be discarded.

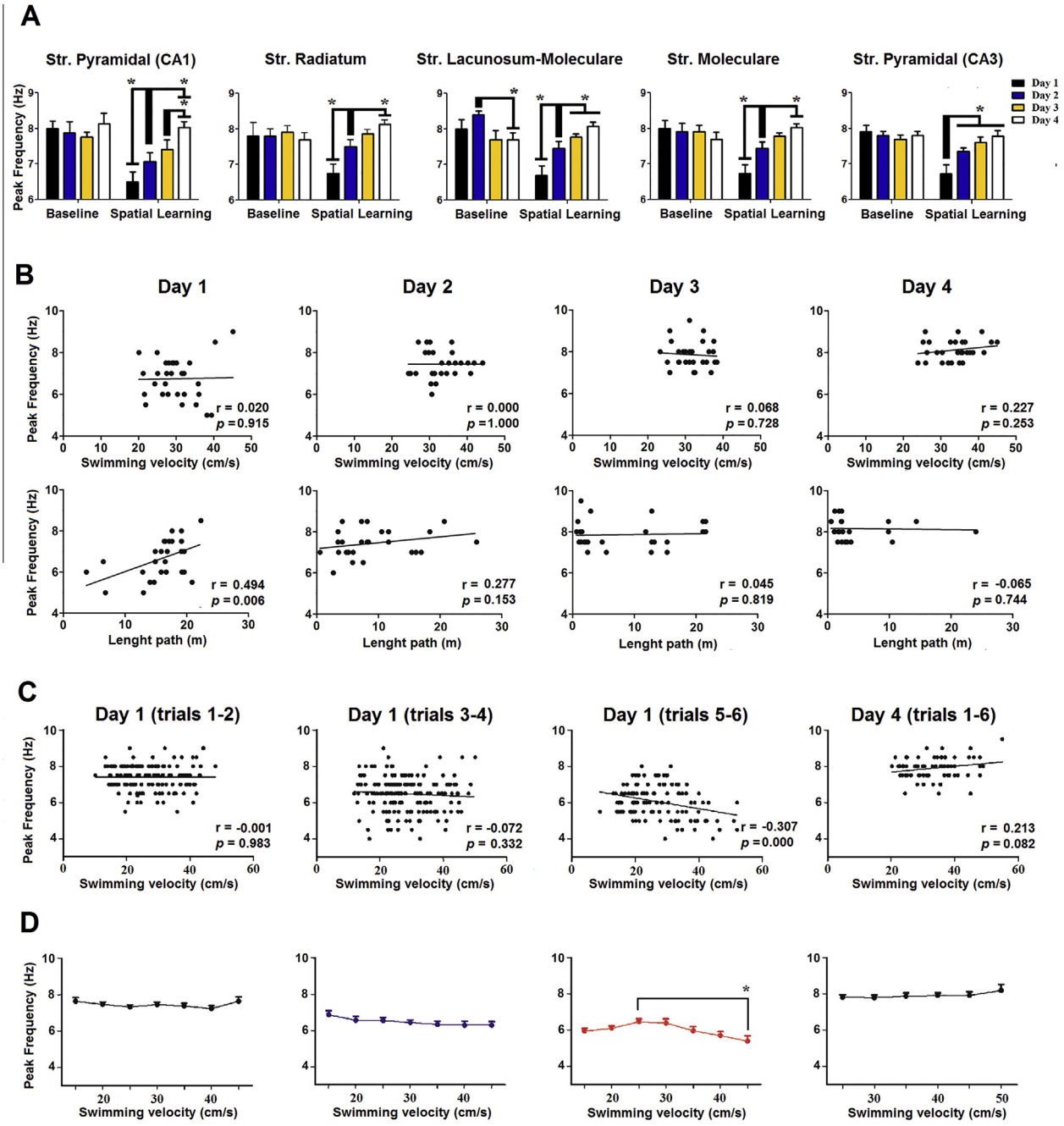

Fig. 4. (A) Progressive increase in peak theta frequency during the spatial learning task across training days in the five hippocampal strata. Mean ± SEM. $^{*}p < 0.05$. (B) Pearson's correlation between peak frequency and swimming velocity (above) or distance traveled (below) on each day of training in the Morris water maze. (C) Pearson's correlation between peak frequency and swimming velocity in epochs of two seconds were analyzed in blocks of two trials on day one (1–2, 3–4, 5–6) as well as in a block of six trials on day four (1–6). (D) Theta frequency at different swimming velocity intervals from blocks of two trials (1–2, 3–4, 5–6) on day one and a block of six trials on day four. Mean ± SEM. $^{*}p < 0.05$.

Modification of the RP at different frequencies of the theta band during spatial learning

The theta band has been described in a wide band range from 4 to 12 Hz; however, two distinct types of hippocampal theta rhythms have been proposed. Theta type 1 is usually composed of fast frequencies (6–10 Hz), is atropine-resistant, and appears during voluntary movement and REM sleep. Theta type 2 is atropine-sensitive, frequently characterized by a slow frequency (5–8 Hz), and appears during immobility and during sensory stimulation under anesthesia with urethane (Kramis et al., 1975; Robinson, 1980). Therefore, to detect whether some specific frequencies predominate—either slow or fast theta oscillations during the spatial learning process—the absolute theta band (4–12) was transformed to relative values in bins of 0.5 Hz. Calculating the relative contribution of each 0.5 Hz to the theta band will provide us information about

what frequencies occurred in the hippocampal networks during the learning process.

To determine changes in RP in frequencies of the theta band (4–12) across training days, a two-way ANOVA for repeated measures (day × frequency) was made for each stratum and condition (baseline and spatial learning). The baseline condition showed a significant interaction of the factors day × frequency in two strata, the sm ($F48,204 = 1.794$, $p = 0.0028$) and the sp (CA3) ($F48,204 = 1.761$, $p = 0.\ 0.0037$) (Fig. 5). The spCA1, sr and slm strata did not show changes in RP across the days. The sm and spCA3 post hoc analysis revealed that in sm, the frequencies 7.5, 8, and 8–8.5 on days two, three and four, respectively, showed low RP in comparison to day one ($p < 0.05$), whereas the spCA3 showed a decreased RP in 7.5 to 8 Hz on day four with respect to day one ($p < 0.05$) (Fig. 5). Thus, in sm and spCA3, there were no changes in frequencies with the maximum power in RP, and the higher RP was maintained at the same frequencies throughout the days. However, a reduction in RP in the frequencies with maximum power was found across days.

The analysis of theta oscillations during the spatial learning task showed changes in the RP of the theta frequencies across the training days in all strata analyzed (two-way ANOVA for repeated measures): spCA1 ($F48,153 = 3.221$, $p < 0.0001$), sr ($F48,204 = 4.909$, $p < 0.0001$), slm ($F48,204 = 5.560$ 6.373, $p < 0.0001$), sm ($F48,204 = 5.470$, $p < 0.0001$), and spCA3 ($F48,\ 204 = 3.466$, $p < 0.0001$) (Fig. 5). The post hoc test revealed a differential distribution in the RP of theta frequencies across the training days from one to four, which was characterized on day one by an increased RP in slow frequencies (5.5–6.5 Hz) in the spCA1, sr, sm and sml strata and by 5.5 Hz for the spCA3 stratum ($p < 0.05$) with respect to day four. However, on day four, the fast frequencies expressed the most RP: 7.5–8.5 Hz in spCA1, 8–9 Hz in sr, sml and sm, and 8.5–9 in spCA3, in comparison to day one ($p < 0.05$) (Fig. 5). Therefore, on day one (when the acquisition of the task was happening), a predominance of lower RP frequencies was found, and this pattern was changing across the training days in a progressive way. Thus, the lower frequencies were decreasing in RP, while the fast frequencies were gradually increasing in RP from days two to four. The most obvious changes occurred in the dendritic strata (sr, sml, sm), while the theta oscillations recorded in the spCA3 also reacted with a modification in RP across the training days, although those changes were less prominent (this can be observed from the distribution of RP at different theta band frequencies) (Fig. 5).

Differential RP distribution in the relationship of baseline-spatial learning across training days

To find changes in theta frequencies associated with spatial learning that overcome the baseline activity, the difference in RP (for each rat) that results from subtracting the daily baseline theta activity from the theta activity recorded during the performance of the water maze task was obtained (Figs. 6 and 7). The theta frequencies with positive values correspond to a predominance of RP for the spatial learning condition over the baseline condition; negative values indicate the predominance of RP of the baseline condition over the spatial learning condition; and values near zero represent no predominance for any one condition.

Day one of training for the spatial learning was characterized by a predominance of slow frequency RP, together with a reduction in fast frequency RP with respect to the baseline condition (95% confidence interval analysis from zero) (Figs. 6 and 7). On day four, the slow frequency RP component was reduced, and fast frequencies increased in RP with respect to the baseline (95% confidence interval analysis from zero) (Figs. 6 and 7). Therefore, two patterns of theta oscillations can be found to be expressed in all hippocampal strata, a slow theta predominant in the early spatial learning and a fast theta present at the end of the learning task, which may correspond to the retrieval state. These results show that the relationship between slow frequency and fast frequency RP was changing across the training days of spatial learning.

To compare the change in frequency distribution across the training days, a two-way ANOVA for repeated measures (factors, frequency and day) was made for each stratum. A significant interaction was found in the five strata: spCA1 ($F48,153 = 4.7371$, $p < 0.0001$), sr ($F48,204 = 6.212$, $p < 0.0001$), slm ($F48,204 = 5.560$, $p < 0.0001$), sm ($F48,204 = 6.838$, $p < 0.0001$), and spCA3 ($F48,204 = 5.436$, $p < 0.0001$) (Figs. 6 and 7). The post hoc analysis revealed that the difference in distribution of RP between the baseline and spatial learning conditions in theta frequencies gradually changed during the successive days of training. The RP in fast frequencies was progressively increased, while the slow frequency RP was decreased. This pattern was reversed on day four compared to day one ($p < 0.05$) (Figs. 6 and 7). The slow theta frequencies predominated at day one in all strata analyzed, while the fast frequencies were reduced in RP with respect to the baseline condition at the time the animal was possibly acquiring information to solve the task. On the other hand, the fast theta frequencies predominated on day four of training, when the animal had successfully learned the task. Days two and three of training showed an RP distribution intermediate between days one and four (Figs. 6 and 7) and on days two and three of the task.

On the other hand, this result showed stratum-specific oscillatory activity because the oscillations in the pyramidal strata (CA1 and CA3) did not show changes in RP distribution between days two and three, while in the dendritic strata (sr, sml, and sm), the RP distribution of theta frequencies was different (Figs. 6 and 7).

Coherence results

To assess how the intra-hippocampal circuits are interacting during the learning process, we evaluated the functional coupling in theta frequencies between the hippocampal strata by analyzing the coherence. The coherence analysis was conducted in two different

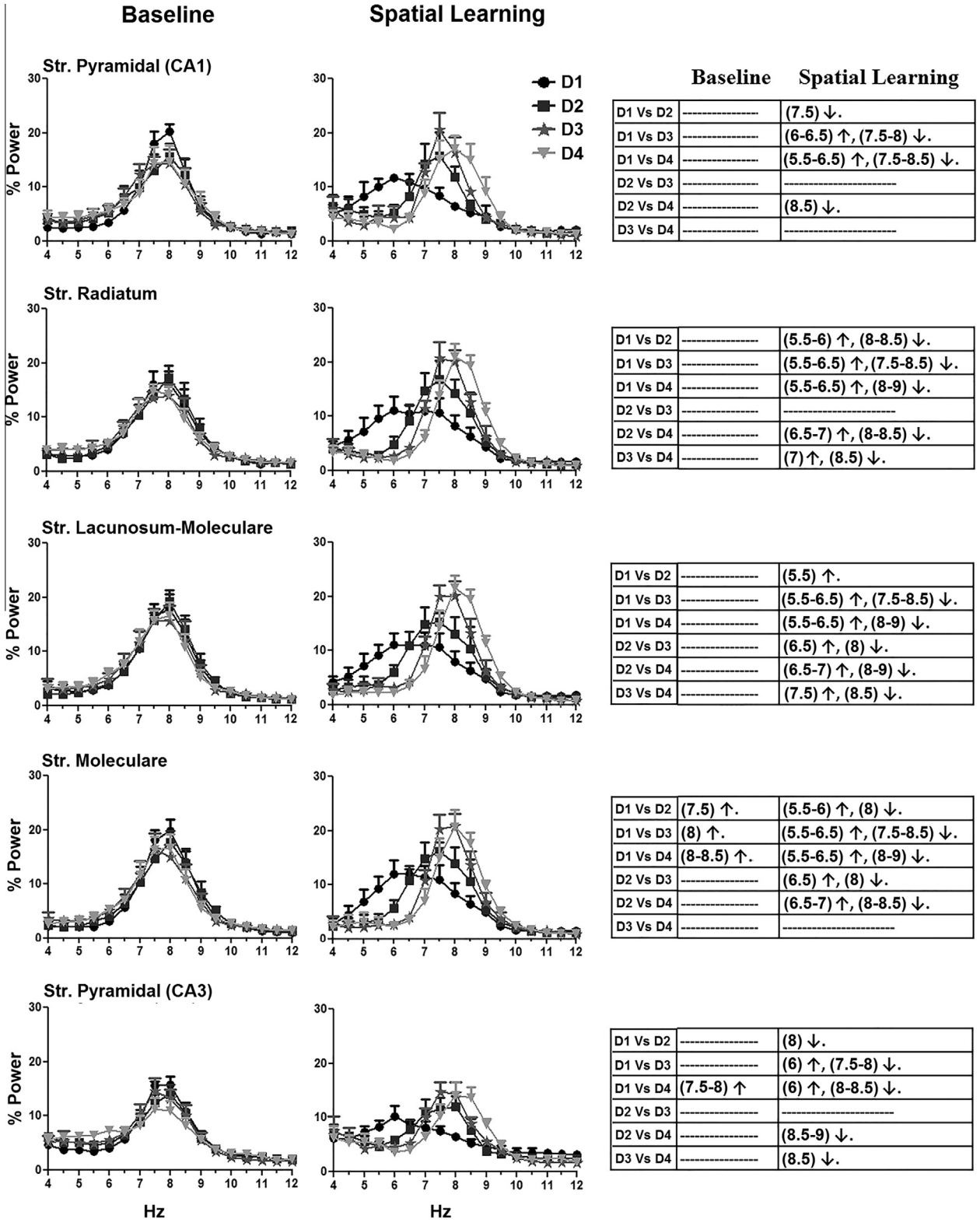

Fig. 5. Distribution of relative power for the frequencies of the theta band recorded in the intra-hippocampal circuits in baseline and spatial learning conditions on the four training days of the Morris water maze task. D = Day. Mean ± SEM. (↓ reduction and ↑ increase = $p < 0.05$.)

conditions, during spatial learning and baseline conditions, across the training days of the task. The comparisons (two-way ANOVA for repeated measures, day and frequency factors) showed significant changes for the spatial learning condition but not for the baseline condition (Fig. 8).

The spCA1 and sm coupling displayed changes across training days ($F42,180 = 2.087$, $p < 0.0005$),

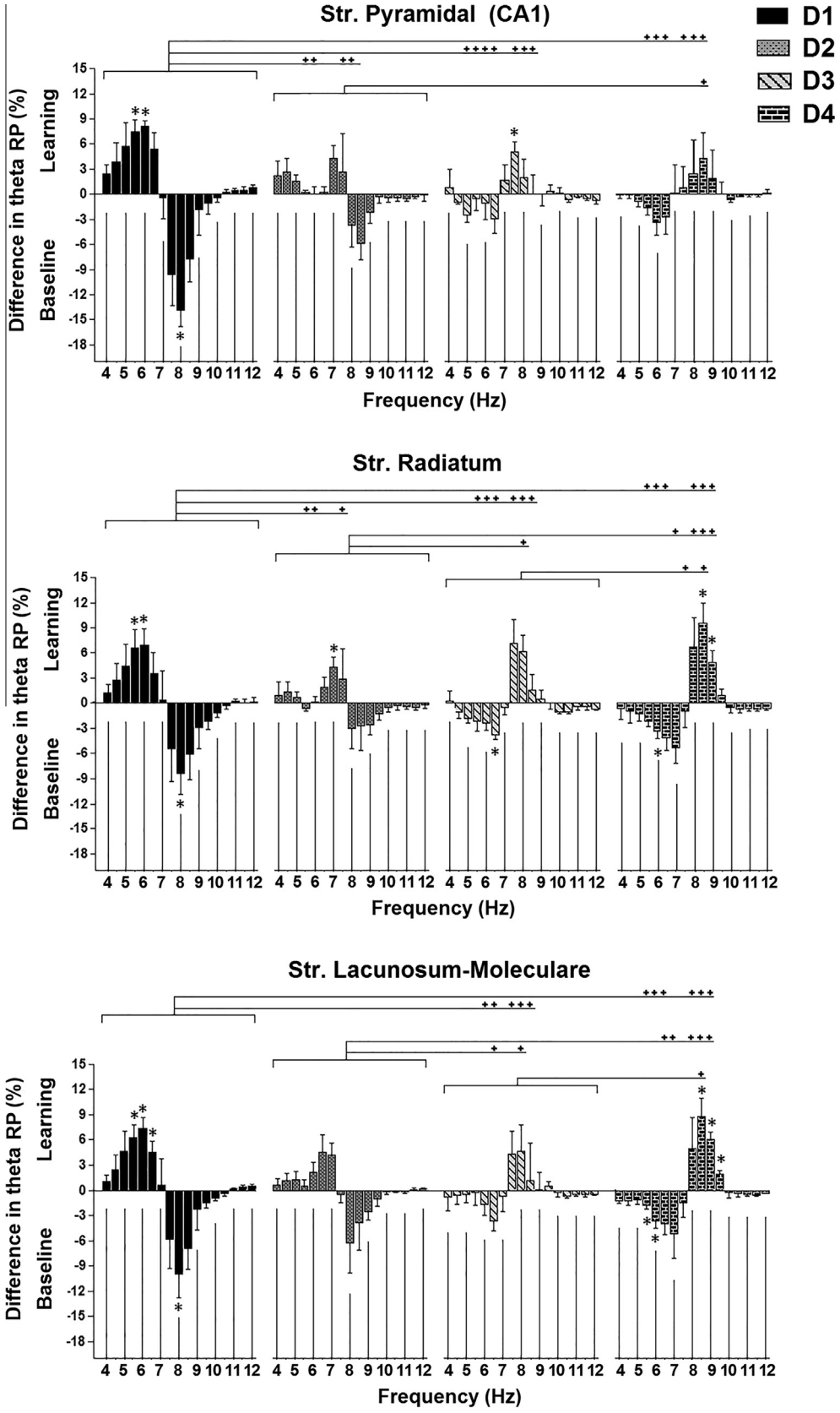

Fig. 6. Difference between spatial learning and baseline RP across the training days for the spCA1, sr, and sm strata. The positive values represent the predominance in RP for the frequencies present in the spatial learning condition, while the negative values indicate the predominance in the RP of frequencies present during the baseline condition, with values near zero representing no change in frequency between conditions. D = Day. Mean ± SEM. ($^{+}p < 0.05$), ($^{*}p < 0.05$ from zero with 95% confidence interval analysis.)

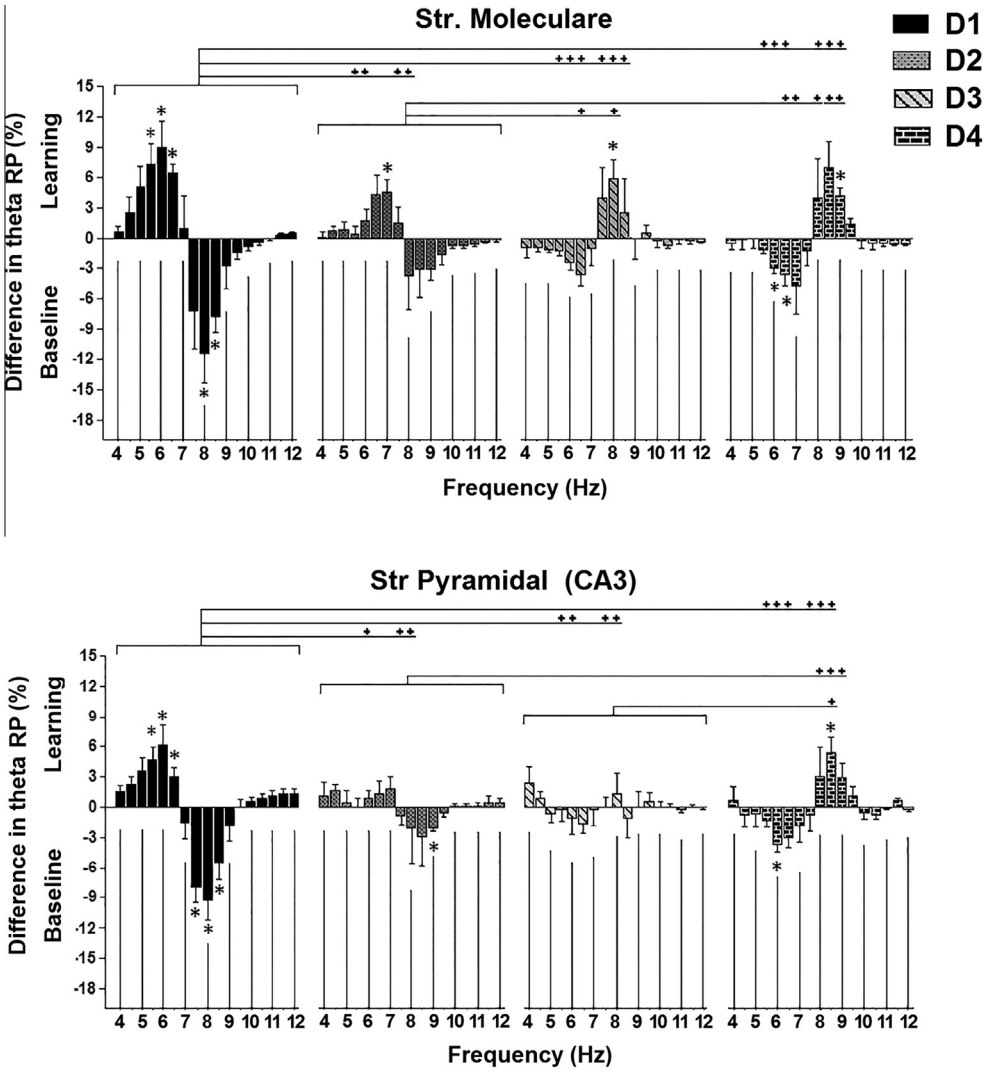

Fig. 7. Difference between spatial learning and baseline RP across training days for the sm and spCA3 strata. The positive values represent predominance in RP for the frequencies present in the spatial learning condition, while the negative values indicate the predominance in the RP of frequencies present during the baseline condition, and values near zero represent no change in frequency between conditions. D = Day. Mean ± SEM. ($^{+}p < 0.05$), ($^{*}p < 0.05$ from zero with 95% confidence interval analysis.)

while increased spCA3 coupling was observed with all strata analyzed: sr ($F42,225 = 2.748$, $p < 0.0001$), sml ($F42,225 = 1.497$, $p = 0.0341$), sm ($F42,225 = 1.845$, $p = 0.0025$), and spCA1 ($F42, 180 = 1.624$, $p = 0.0160$) (Fig. 8). The coherence of sr with slm ($F42,225 = 2.704$, $p < 0.0001$) and sm ($F42,225 = 3.397$, $p < 0.0001$) changed across training days of the spatial learning task (Fig. 8).

The post hoc analysis showed that the spCA1 coherence with sm was high for slow frequencies on day one with respect to days two (6 Hz), three (6 Hz) and four (5.5–6 Hz), while the fast frequency coherence increased on day four (8.5 Hz) with respect to day one ($p < 0.05$). The spCA3 showed increased coupling with all strata in fast frequencies on days three and four of training with respect to day one. This increase in coherence values may suggest that the CA3 network is actively interacting (direct or indirectly) with another intra-hippocampal circuit when the rat is retrieving information already learned (Fig. 8).

Specifically, the comparison of spCA3-spCA1 coherence showed higher values on days three (8–9 Hz) and four (8.5–9.5 Hz) compared with day one. The coherence between spCA3 and sr was higher in the 8.5–9.5 Hz frequencies on day three and in the frequencies 8–10 Hz on day four with respect to day one. The coherence of spCA3 with sml and sm showed a similar increasing pattern of values for the high frequencies on days three (8–8.5 and 8–9 Hz in sml) and four (8–9 and 8.5 Hz in sm) compared with day one ($p < 0.05$) (Fig. 8).

The coupling of the sr theta frequencies with sml and sm also changed during spatial learning. The coherence between sr and sml was higher for the slow frequencies on day one with respect to day four and higher for fast frequencies on days three and four with respect to day one. The coherence between sr and sm was higher for slow frequencies on day one compared with days three and four and higher for fast frequencies on days two, three and four with respect to day one. The high

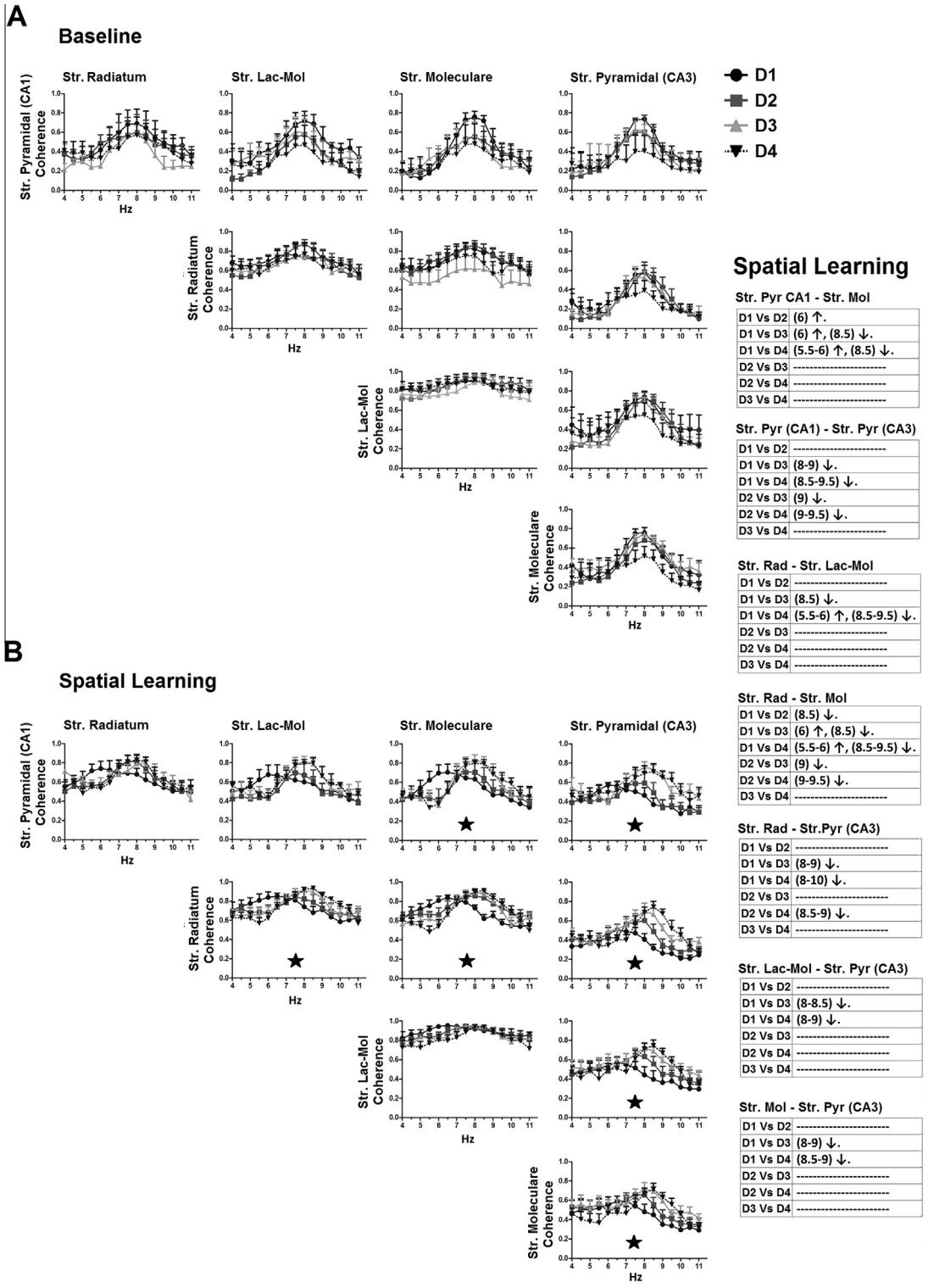

Fig. 8. Coherence theta values of baseline (A) and spatial learning (B) conditions across the training days of the task. D = Day. Mean ± SEM. (↓ reduction and ↑ increase = $p < 0.05$.) The star symbol represents statistical significance of two-way ANOVA for repeated measures (day and frequency factors).

coupling to slow theta frequencies found on day one between the dendritic strata (sr with slm and sm) strengthens the notion that the processing and handling of new information (to navigate a complex environment) may be established through a reduction of the oscillatory frequency of the theta rhythm.

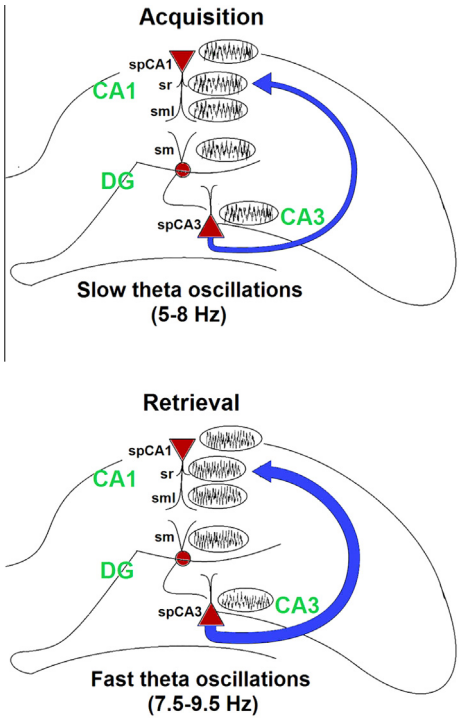

Fig. 9. Schematic representation of oscillatory activity theta in the hippocampal circuits during different states of processing of spatial information from acquisition to retrieval. The retrieval state is characterized by an increased coupling between the CA3 and CA1 areas.

DISCUSSION

It was determined that the oscillatory activity in the hippocampal networks changes over the course of training in the Morris water maze; these changes in network activity might underlie navigational learning. The results show that the early acquisition stage of spatial learning was characterized by a slow frequency theta rhythm ranging from 5.5 to 8 Hz and high coupling between specific hippocampal strata on slow frequencies. The gradual establishment of learning across of the training days was accompanied by a gradual increase in coherence and RP for fast theta frequencies. The last day of the Morris maze training was characterized by a higher coupling between the spCA3 and other hippocampal strata at fast frequencies (7.5 to 10 Hz) (Fig. 9).

Slow frequency theta associated with early acquisition of spatial information

With respect to the reduction in frequency found on day one, previous reports have shown a reduction in theta frequency during the exploration of a novel environment on a standard foraging task in an open field (Jeewajee et al., 2008; Wells et al., 2013) and when rats alternated in a novel linear track (Penley et al., 2013). However, of the previous works, Wells et al. (2013) took into account the well-established relationship between theta frequency and running speed and found that the reduction in frequency during environmental novelty was associated with a flattening in the frequency-speed slope. This phenomenon was interpreted as an underestimation of spatial metrics in the novel environment (Wells et al., 2013). The environmental novelty in Wells et al. (2013) and Jeewajee et al. (2008) showed a reduction in peak frequency that did not fall below 8 Hz, while in the present results, the reduction in peak frequency ranged from 6.5 to 6.8 Hz on day one (Fig. 4). These differences may be attributed to different processes involved in both paradigms because the exploration of a novel environment implies a passive coding of spatial information. However, the early acquisition of spatial learning in the water maze requires the coding and use of new spatial information acquired on the same day one in order to find navigational strategies to solve the task.

To assess whether changes in frequency can be explained by unspecific modifications in theta activity across the training days, a baseline movement-associated theta was analyzed and compared with respect to the theta activity recorded during spatial learning. The results showed that the baseline theta was constant across the training days, and the changes in coherence and frequency were only established in the spatial learning condition. On the other hand, a characteristic of the theta oscillations is their positive relationship between frequency and amplitude with the animal's locomotion speed (Vanderwolf, 1969; McFarland et al., 1975; Jeewajee et al., 2008; Hinman et al., 2011; Wells et al., 2013). This relationship has been used for models to explain the coding of spatial metrics as animals traveled a familiar environment (Burgess et al., 2007; Burgess, 2008). However, the present results do not show a relationship between swimming velocity and theta frequency during navigational learning (Fig. 4), and there were no changes in the swimming velocity across the training days of the task, while the theta frequency and coherence were dynamically changing during the different stages of navigational learning. These results are in accordance with previous observations, which have found that the relationship between theta frequency and running velocity is uncoupled or decreased when the animal is encoding behaviorally relevant information, such as during spatial learning decision-making and novelty (Jeewajee et al., 2008; Montgomery et al., 2009; Schmidt et al., 2013; Wells et al., 2013; Belchior et al., 2014). In this sense, the network activity in the theta frequency in the hippocampal circuits may show changes related to diverse cognitive processes instead of an exclusive relationship with speed of movement.

Modifications of theta oscillations during spatial learning

The modifications in theta activity observed in the present work are consistent with previous reports, in which spatial learning (Olvera-Cortés et al., 2002, 2004; DeCoteau et al., 2007; Benchenane et al., 2010) and spatial decision-making (Jones et al., 2005; Montgomery et al., 2009; Schmidt et al., 2013; Belchior et al., 2014) are characterized by changes in the theta oscillations or their coupling, suggesting a role of these oscillations in the cognitive process. Moreover, pharmacological modifica-

tions, such as a reduction in the serotonin levels of the hippocampus (Gutiérrez-Guzmán et al., 2011) or medial septum (López-Vázquez et al., 2014), that result in a facilitation of learning are characterized by the promotion of an early switch from slow to fast theta frequencies across training days. However, alterations in the dynamics of theta oscillations (characterized by a modification in the switch from slow to fast frequencies) during spatial learning induced by a serotonin reduction in the supramammillary nucleus (Hernández-Pérez et al., 2015) or by a combined reduction in the supramammillary/posterior hypothalamus nuclei (Gutiérrez-Guzmán et al., 2012) resulted in impaired spatial learning, while the injection of chlordiazepoxide (CDP) into the supramammillary nucleus produced a modest decrease in theta frequency and a modest impairment of spatial learning (Pan and McNaughton, 1997). Additionally, the differential performance of place learning in the water maze between young and old rats has been related to differential oscillatory theta activity, with better performance in young rats compared with a predominant theta of fast frequencies (Olvera-Cortés et al., 2012). Therefore, it seems that a modification in frequencies of theta oscillations may be related to distinct aspects of spatial learning. In this sense, we start from the notion that in the same behavioral stage (walking, running, or swimming), the network pattern may be changing as a consequence of information-processing performance from moment to moment. The capacity of intra-hippocampal circuits to perform different computational operations such as encoding, consolidation and retrieval of mnemonic information in the same network may require dynamic modification of oscillatory patterns. Therefore, according to the present results, it is possible to suppose that the encoding of behaviorally relevant information might be processed during a hippocampal decreased theta frequency period, while the retrieval stage might be signaled for a restoration and/or increase in theta frequency with respect to the movement-associated theta recorded in the baseline condition, along with an increase in the coupling between intra-hippocampal areas (this topic will be discussed in detail below). In agreement with this view, recent models and experimental data suggest that a reduction in theta frequency can help encode environmental novelty (Barry et al., 2012a, 2012b). The mechanism proposed suggests that the high cholinergic tone generated for environmental novelty might decrease the frequency of resonance in the theta band of stellate cells (Heys et al., 2010), which in turn could result in altered properties of grid cells, such as increased field size, decreased regularity and a reduction in the intrinsic frequency of the theta modulation of firing, as has been shown in spatial novelty (Barry et al., 2012a). The reduction in intrinsic frequency is a factor that might be involved in the reduction in frequency of the oscillatory activity on a network level (Barry et al., 2012b). Thus, it is possible to assume that the reduction in theta frequency found in navigational learning can be a propitious network state for encoding, organizing and handling new information that has not yet been consolidated.

In addition, other non-cognitive aspects associated with the Morris maze task, such as high arousal levels, stress, and emotional content (Vorhees and Williams, 2014), that accompany the learning process may have some influence over the theta activity (Sainsbury and Montoya, 1984; Sainsbury et al., 1987; Bland et al., 2006; Tendler and Wagner, 2015). For example, it has been shown that negative arousal states are associated with sensitive-atropine theta (Sainsbury and Montoya, 1984; Sainsbury et al., 1987) and that the recall of a fearful memory elicits theta oscillations of slow frequency during periods of movement in the rat (Tendler and Wagner, 2015). Therefore, we do not discard the possibility that changes in the neuromodulator tone related to the expression of non-cognitive aspects that accompany the spatial learning may be tuning the oscillatory dynamic of the network in order to provide a suitable state for information processing. In that sense, the high cholinergic activity expressed on day one of the Morris maze (Hosseini-Sharifabad et al., 2011), probably associated with high arousal and encoding of novel information, may contribute to the theta activity expressed on that training day.

The acquisition and retrieval of spatial information are differentially encoded by theta oscillation in the intra-hippocampal circuits

Navigational learning may be achieved through two interlinked mechanisms known as path integration and map-based allocentric navigation. The first integrates information about linear and angular self-movement, allowing the animal to return to its home using the shortest routes (Mittelstaedt and Mittelstaedt, 1980), while the second requires environmental signals and their relationships for the animal to determine its position within a spatial environment (O'Keefe and Nadel, 1978). It has been shown that when the external landmarks are reduced, the navigation may be achieved through path integration. However, if the environment signals are available, the navigation may integrate the external signals to update the spatial representation (Etienne et al., 1985). Both systems involved in the navigation work together when the external signals are available, and it has been proposed that the hippocampus and entorhinal cortex support some aspect of these navigational strategies (McNaughton et al., 2006a; Barry and Burgess, 2014). Moreover, Buzsáki and Moser (2013) have hypothesized, according to experimental dates based on specific firing patterns and oscillatory dynamics in theta cycles, that the same neuronal algorithm in the entorhinal cortex and hippocampus may support self-reference navigation and episodic memory. To successfully navigate the Morris maze, the animal has to form a spatial map by building an internal representation of the relationships between the distal signals (Vorhees and Williams, 2014). In addition, the self-movement proprioceptive information encoded in the entorhinal cortex and hippocampus during the spatial behaviors (Etienne et al., 1985; Buzsáki and Moser, 2013; Barry and Burgess, 2014) may continually help the animal update its position during displacement

in the Morris maze. Therefore, due to the task's complexity, navigational learning requires a gradual process of consolidation across training days.

Because day one signaled slow theta frequencies and the later training days showed a progressive increase in frequency, it is suggested that this increase can be interpreted as a change in computational operations (from encoding to retrieval). Because the information required to solve the task is gradually consolidated across training days, on the last days of training, the hippocampal networks do not require work at slow frequencies (encoding state); instead, a retrieval stage may predominate. Hence, the fast frequencies might be a propitious network state for the retrieval and handling of information previously stored. In other words, the slow theta frequencies might be a favorable state to encode information, while fast frequencies may support the retrieval of information previously consolidated in the cortico-hippocampal system.

Differential coupling of the intra-hippocampal networks during different stages of spatial learning

It is known that the CA1, CA3 and DG areas contribute differently to distinct aspects of spatial learning. Thus, the theta oscillations in each stratum, as well as their coupling, can reflect activity patterns associated with different stages of spatial learning in a way specific to the involved networks. The analysis of the coherence between hippocampal strata reveals that the coherence values in the CA1 area did not change in the total of the theta band between their own strata, or with respect to the molecular stratum of DG, across training days. However, a detailed analysis showed a preferential coupling for slow or fast theta frequencies during navigational learning: spCA1-sm, sr-slm, and sr-sm exhibited higher coherence for frequencies ranging from 5 to 6.5 Hz on day one with respect to days three and/or four and a higher coherence on days three and/or four for fast frequencies from 8 to 9 Hz with respect to day one. The higher coupling between dendritic strata (sr-slm, sr-sm) in slow frequencies during the early acquisition stage and in fast frequencies during the late learning stage suggests a dynamic coupling between specific elements of hippocampal circuits. Moreover, there are no direct connections between them, and this coupling can originate from a different source that enables several dendritic strata to work in a coordinated way with slow or fast frequencies depending on the learning stage. The cortical and subcortical inputs as well as the intrinsic connectivity are possible candidates to promote such coordination. Because theta oscillations have been observed along the entorhinal-hippocampal system (Buzsáki et al., 1983; Schomburg et al., 2014), and because the theta-coordinated neural activity of the entorhinal cortex predicts the timing of the theta current sink in the target strata in the hippocampus (Mizuseki et al., 2009), the changes observed in the theta activity might involve upstream regions such as the entorhinal cortex. In this sense, different aspects of spatial information can be encoded by different types of cells, such as grid cells, head direction cells, and border cells (Moser et al., 2015) found in the entorhinal cortex, which in conjunction provide the spatial metric to encode the animal's position within an environment. In addition, the proximodistal organization of the entorhino-hippocampal system determines the spatial properties encoded by CA1 cells (Henriksen et al., 2010), while a strong coordination between entorhinal inputs arriving at CA1 proximal locations (i.e., closer CA3) has also been observed (Laurent et al., 2015), suggesting that the entorhinal-hippocampal system displays exquisitely organized activity at the proximodistal axis (Inostroza et al., 2013). Therefore, changes in the coupling found in the present work might require the participation of specific pathways through the entire entorhinal-hippocampal system that allow the coordination of different hippocampal dendritic strata.

Furthermore, the hippocampal theta activity is influenced by a group of subcortical nuclei that form part of the synchronizing ascending system (SAS), which includes the mesencephalic and diencephalic relays that reach the medial septum and hippocampus. Additionally, the coordination of widely distributed networks may be originated, in part, by the SAS (Bland et al., 1990; Kirk et al., 1996). Moreover, the medial septum is a relay necessary for the generation of the hippocampal theta (Petsche et al., 1962; Mitchell et al., 1982) for hippocampal-dependent learning and memory (Winson, 1978; Mizumori et al., 1990), and an increase in coherence has been shown between the medial septum and hippocampus during spatial learning in the Morris maze (Hernández-Pérez et al., 2015). Furthermore, a decrease in coupling between the medial septum and hippocampus produced by a reduction in serotonin in the supramammillar nucleus coincides with inefficient task performance (Hernández-Pérez et al., 2015). Therefore, the medial septum may be a key area for the coordination of distinct hippocampal strata, working as a pacemaker that coherently entrains hippocampal networks. Because the coherent oscillations reflect or enable effective communication, the coordinate state in dendritic strata could favor the integration and organization of information during different computational operations such as encoding and retrieval.

Interestingly, the spCA3 coherence increased (on fast theta frequencies) in all strata analyzed on the last training days, with the most prominent changes being established with respect to spCA1 and sr. This increase coincided with the improvement of task performance; therefore, it is suggested that the increased synchronization in the CA3-CA1 networks may be involved in the retrieval of spatial information. In agreement with this idea, several studies have shown that the disruption of such a pathway generated serious deficiencies in the retrieval of spatial information (Brun et al., 2002; Nakazawa et al., 2002; Florian and Roullet, 2004; rolls and Kesner, 2006). Furthermore, during the performance of an alternation task in a T maze, an increase in power and coherence in the gamma band in the CA3-CA1 network was reported during the portion of the maze associated with the retrieval of information from a previous experience (Montgomery and Buzsáki, 2007).

Because the gamma rhythm is nested (Bragin et al., 1995; Canolty et al., 2006; Colgin et al., 2009) in the theta phase, it is possible that the dynamic interplay between theta and gamma rhythms may participate in the retrieval process.

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