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Serotonin depletion of supramammillary/posterior hypothalamus nuclei produces place learning deficiencies and alters the concomitant hippocampal theta activity in rats

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ABSTRACT

Hippocampal theta activity is important for the acquisition of spatial information and is strongly influenced and regulated by extra-hippocampal inputs from the synchronising ascending system (SAS), which includes the supramammillary nucleus (SUMn) and the posterior hypothalamic nucleus (PHn). Together these nuclei play an important role in controlling the frequency encoding of theta activity and are innervated by serotonin synapses, which also regulate theta activity and learning abilities. The participation of the SUMn in place learning and modulation of hippocampal theta activity were recently shown; thus, we questioned whether serotonin acting on SUMn/PHn could modulate place learning ability and concurrent hippocampal theta activity. The serotonergic terminals of the SUMn/PHn in rats were lesioned through 5,7-dihydroxytryptamine (5,7-DHT) infusion, and hippocampal theta activity during the Morris water maze test was recorded. Rats in the vehicle group learned the task efficiently and showed learning-related theta changes in the CA1 and dentate gyrus regions throughout the training. The 5-HT-depleted rats were deficient in the Morris water maze task and showed theta activity in the CA1 and dentate gyrus that were unrelated to the processing of learning. We conclude that serotonin can regulate the hippocampal theta activity acting on the SUMn/PHn relay of the SAS and that the influence of 5-HT in these nuclei is required for the learning-related changes in hippocampal theta activity that underlie the successful resolution of the Morris water maze task.

1. Introduction

Hippocampal theta activity is characterised by a slow, almost-sinusoidal rhythmic activity pattern with frequencies of 4–12 Hz that are associated with voluntary behaviours (Bland, 1986; Buhot et al., 1995; Vanderwolf, 1988) and is important for learning and spatial memory (2004; Eichenbaum et al., 1992; McNaughton et al., 2006, Olvera-Cortes et al., 2002).

Hippocampal theta activity is regulated by extra-hippocampal inputs, that form part of the synchronising ascending system (SAS), a multi-synaptic circuit that includes structures from the brainstem, caudal diencephalon (posterior hypothalamic nucleus, PHn and supramammillary nucleus, SUMn), and septum (Vertes and Kocsis, 1997). Collateral projections of SUMn neurons that reach the medial septum/vertical limb of the diagonal band of Broca (MS/vBDB) and the hippocampal formation (Vertes and McKenna, 2000), together with projections of the PHn to the medial septum (Vertes, 1992) play an important role in controlling the frequency encoding of theta activity (Kirk and McNaughton, 1993). Briefly, electrical stimulation or the application of carbachol in the PHn produces continuous

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theta activity in the hippocampus, together with the activation of theta-ON cells in the MS/vBDB (Bland et al., 1990; 1994; Oddie et al., 1994; Smythe et al., 1991); in contrast, the electrolytic lesion of the PHn attenuates the hippocampal theta activity and reduces the theta frequency (Robinson and Whishaw, 1974). Additionally, the injection of procaine into the PHn blocks the hippocampal theta activity generated by reticular stimulation (Oddie, et al., 1994) and the septal theta-related phasic cell discharge (Bland, et al., 1994). Similarly, the SUMn stimulation produces hippocampal theta activity (Oddie, et al., 1994; Vertes, 1981), whereas the injection of procaine into several SUMn sub-regions in anesthetised rats reduces the frequency and amplitude of theta activity generated by the stimulation of the *reticular pontis oralis* nucleus (Kirk and McNaughton, 1993).

In spite of the influence of the SUM/PHn on hippocampal electrical activity, few studies have evaluated the participation of the SUM and any of the PHn, in memory. Functionally, the inactivation or pharmacological manipulation of the SUMn alters place-learning ability and concurrent hippocampal theta activity. Briefly, the reversible inactivation of the SUMn by the infusion of lidocaine impairs both the consolidation of reference memory and the consolidation and retrieval of working memory, evaluated in the Morris water maze (Shahidi et al., 2004). In addition, the injection of chlordiazepoxide into the SUMn produces a modest impairment in a one-day place-learning test in the Morris maze, and is associated with a decrease in the hippocampal theta frequency (0.35–0.5 Hz) (Pan and McNaughton, 1997). Thus, the SUMn participates in associative memory processing possibly by modulating hippocampal excitability.

In contrast, serotonin (5-HT) desynchronises hippocampal theta activity. The stimulation of the medial raphe nucleus desynchronises the hippocampal EEG (Assaf and Miller, 1978; Kitchigina et al., 1999; Vertes and Martin, 1988; Vinogradova et al., 1999), and lesions to the medial raphe nucleus resulted in the presence of persistent and continuous trains of theta activity (Maru et al., 1979; Vertes, 1986; Vinogradova et al., 1999). Additionally, 5-HT modulates learning and memory processes. Reductions of cerebral or hippocampal 5-HT have produced both learning enhancements or deficiencies or have had no effect on learning tests (1990; Adams et al., 2008, Altman et al., 1984; Anguiano-Rodriguez et al., 2007; Buhot et al., 2000; Riedel et al., 1999; Volpe et al., 1992), whereas an increase in 5-HT activity resulted in deficiencies in learning tests (Buhot et al., 1995; Carli et al., 1995; Riedel et al., 2002). However, it is still not known whether a relationship exists between serotonin manipulations, learning and hippocampal theta activity.

Recently, it was shown that significant, selective 5-HT depletion in the hippocampus that produced the prevalence of high-frequency theta activity (6.5–9.5 Hz) in CA1 facilitates spatial learning during the resolution of the Morris water maze (Gutierrez-Guzman et al., 2011). Because SUMn/PHn constitutes a critical nodal area in the encoding of the amplitude and/or frequency of theta activity (Bland et al., 1990, 1994; Kirk and McNaughton, 1993), SUMn contributes to associative memory modulation and both nuclei are innervated by serotonin (Vertes and Kocsis, 1997; Vertes and Martin, 1988), we questioned whether serotonin acting on SUMn/PHn could modulate place learning ability and concurrent hippocampal theta activity. Serotonergic terminals of the SUMn/PHn were lesioned in rats through a 5,7-dyhidroxytriptamine infusion, and hippocampal theta activity was recorded during performance of the Morris water maze test.

2. Materials and methods

2.1. Animals

Twenty-six Sprague–Dawley male rats weighing 350–450 g were used. All 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 approved by the Research Ethics Committee of the Instituto Mexicano del Seguro Social. The rats were housed under standard conditions in the animal facility. The rats were assigned to the vehicle group or to the SUMn/PHn serotonin depleted group (5HT-D).

2.2. Surgery

For the implantation of the recording electrodes, rats were first given an injection of atropine sulphate (0.1 mg/kg i.p.), followed 10 min later by an injection of pentobarbital (30 mg/kg i.p.). A concentric bipolar electrode was chronically implanted into the CA1 field of the right hippocampus at the edge of the strata oriens and pyramidal layer, with the following coordinates: 4.0 mm posterior to the bregma, 2.2 mm lateral from the midline, and 2.6–2.7 mm dorsoventral from the cranial surface. A second electrode was placed into the dentate gyrus with the following coordinates: 3.5 mm posterior to the bregma, 1.5 mm lateral from the midline and 3.4 mm dorsoventral from the cranial surface (Paxinos and Watson, 1989). A third recording electrode was implanted into the SUMn with the following coordinates: 4.7 mm posterior to bregma, 0.2 mm lateral from the midline and 8.7 mm dorsoventral from the cranial surface (the data obtained from this electrode were not included in the present work).

The recording electrodes were made of nichrome wire with a diameter of 65 μm fastened inside a stainless steel # 25 calibre cannula isolated with epoxy resin, with a small surface exposed on the tip. A stainless steel scroll placed on the bone over the frontal cortex surface served as the ground. The implant was fixed with acrylic cement.

The 5HT-D group of rats received an injection of desipramine (30 mg/kg i.p., to protect noradrenergic terminals) 30 min before receiving 2 bilateral injections of 5,7-dihydroxytriptamine (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. The injections were placed into the SUMn (4.7 mm posterior to the bregma, 0.2 mm bilateral from the midline, and 8.7 mm dorsoventral from the cranial surface) and into the PHn (3.7 posterior to the bregma, 0.3 mm bilateral from the midline, and 8.2 mm dorsoventral from the cranial surface). Vehicle rats received injections of the vehicle solution (0.4 μl, at a rate of 0.1 μl/min).

2.3. Behaviour

Rat training began 15 days after surgery. Behavioural tests were conducted using a swimming pool (1.4 m in diameter) filled with water maintained at 28–30 °C and dyed blue with gentian violet. A glass platform (12 cm × 12 cm) was placed inside the pool such that its surface was 1.5 cm below the water level and was maintained in a fixed position at the north quadrant of the pool. The pool was located in an experimental room (5 m × 5 m) with constant, stationary environmental extra-maze cues (e.g., laboratory equipment, experimenters).

The rats were trained for six consecutive days, undergoing four daily trials with inter-trial intervals of 2 min each. Each trial consisted of placing the animal in the water facing the pool wall and removing it 15 s after reaching the platform. If the rat failed to find the platform within 60 s, it was placed on the platform for 15 s. On the seventh day, the platform was removed from the pool and the animals were challenged with a single 30 s search trial (the probe trial). The maze was virtually divided into four equal sized quadrants identified by the cardinal points. For each trial, the rat was randomly placed at different quadrants of the pool and the position of the sunken platform remained constant during the training. The behavioural tests were video-recorded, the paths traced and the lengths estimated. The mean swim distances from the four daily trials were calculated from the latency and distance data. In the probe trial, the number of crosses

made by the rat into the central annulus in each quadrant (corresponding to the platform's position and area) was counted.

2.4. Electrical activity

To record the behavioural training tasks, the rats were connected to EEG equipment (Neurodata acquisition system, GRASS Mod 15, Astro Med Inc. 600 E. Greenwich Ave., W. Warwick, RI 02893, USA) through a male connector attached to a female connector on the head of the rat and to a gyratory commutator (supplied by Plastics one®) through a lead connector. For offline analysis, the hippocampal (CA1 and dentate gyrus) and SUMn EEG were amplified and stored on a hard disk with a sampling frequency of 512 Hz. Bipolar recordings were taken using the Nichrome wire as G1 and the cannula as G2, with the low pass and the high pass filters set to 100 Hz and 1 Hz, respectively. Only the results obtained from the hippocampal EEG are included in the present work.

A 15 s baseline EEG recording was made for each wet rat in its home cage under an immobile alert condition. An EEG was also recorded during the platform search, from the time the rat was placed in the water until the rat reached the platform. Finally, the EEG activity was recorded during the 15 s in which the rat was on the platform. The temporal relationship between each sub-band and the spatial learning were analysed after the entire hippocampal theta frequency band was divided into three sub-bands based on the proposed functional sub-divisions (Vanderwolf, 1988; Vanderwolf et al., 1989; Vinogradova, 1995): low-frequency theta activity (4–6.5 Hz), high-frequency theta activity (7–9.5 Hz), and maximum frequency theta activity (10–12 Hz).

The EEG signal was submitted to Fourier analysis (FFT), using the software EEGmagic (Guevara and Hernández-González, 2009) to obtain the power spectra. The relative power (RP, obtained as the percentage of absolute power of each sub-band) of each sub-band was calculated at each test stage, and the mean values for each day were calculated and compared. A $P<0.05$ was considered significant for all comparisons.

2.5. Immunohistochemistry

After the behavioural tests were completed, the rats were perfused intracardially with a phosphate-buffered washing solution (pH 7.4; 0.1 M), followed by a phosphate-buffered, 4% paraformaldehyde fixing solution. The brains were removed, and a block containing the SUMn and PHn was isolated and 40 μm-thick coronal slices obtained using a microtome (Microm, HM 650 V). Free-floating sections from the two groups were immunostained for 5-HT using the standard avidin–biotin complex (ABC) method and processed simultaneously using the same reagents and incubation times. Briefly, endogenous peroxidase activity was blocked by incubating the slices in 0.6% H_2O_2 for 30 min. Sections were then incubated in 3% normal goat serum (NGS) at room temperature for 30 min and placed in 0.1 M phosphate buffered saline (PBS) containing 35% NSG, 0.5% Triton X-100 and rabbit anti 5-HT (1:1000, Chemicon Intl. Inc.) at 4 °C for 36 h. After several rinses with PBS containing 0.1% Triton X-100, the sections were incubated in anti-rabbit IgG (1:200) with 3% NGS for 2 h at room temperature. The sections were rinsed again and incubated in 0.5% ABC solution for 45 min. Finally the sections were placed in PBS with 0.5% 3,3′-diaminobenzidine (DAB) and 0.1% H_2O_2 for 3 min. The sections were mounted on glass slides using Cytoseal 60®. Each preparation was examined under a light microscope.

Six slices per nucleus per animal were counted, and the anterior, middle and posterior extension of each nucleus were sampled. The number 5-HT immunoreactive fibres was assessed in three areas, bilaterally for each slice and nucleus. The mean number of 5-HT immunoreactive fibres in the three counts and 6 slices was obtained, and the mean number of fibres expressed as number of fibres in a 1 mm^2 area was compared for each nucleus and group. The same slices were also used to verify electrode placement.

3. Results

All rats with misplaced electrodes or without lesions of the serotonergic terminals were not included in the results. Two vehicle rats and two 5-HTD rats were discarded for misplaced electrodes, and six 5-HTD rats were excluded for failed lesion of the SUMn/PHn serotonergic terminals. Thus, the vehicle group included 15 rats, and the 5HT-D group included 11 rats.

3.1. Immunohistochemistry

The number of immunoreactive fibres in a 1 mm^2 area was significantly reduced (*T* test, $P<0.001$) in SUMn and PHn in the 5HT-D group compared to the vehicle group (Fig. 1, bar graph). A micrograph showing the reduction in the number of 5-HT immunoreactive fibres in SUMn and PHn is shown in Fig. 1.

3.2. Behaviour

In the intra-group comparisons of the escape latency (Friedman's ANOVA and Wilcoxon's test), the rats in the vehicle group showed a significant reduction in escape latencies ($X^2=37.952$, $P<0.001$) on day 3 ($P=0.005$) and days 4–6 ($P\leq0.002$) compared to day 1, whereas the 5HT-D group did not show a significant reduction in escape latencies ($X^2=10.584$, $P=0.060$, Fig. 2A). However, there were no significant differences in inter-group comparisons (Mann–Whitney's *U* test; $X^2=2.983$, $P=0.084$).

Distance and swimming velocities were compared using a repeated measures ANOVA and Tukey's test. The vehicle animals recorded shorter swimming distances [$F(5, 54)=14.595$, $P<0.001$] on days three ($P=0.005$), four ($P=0.001$), five and six ($P<0.001$) compared to the first day. Although, the 5HT-D group did not show a significant reduction in distance travelled, the group did show a bias for reduction of distance [$F(5, 50)=2.451$, $P=0.074$] (Fig. 2B). However, no inter-group differences were observed [$F(1, 20)=1.159$, $P=0.295$].

Intra-group comparisons of swimming velocities showed that the vehicle group significantly increased velocity [$F(5, 55)=4.493$, $P=0.002$] on day 4 ($P=0.017$) and day 6 ($P=0.023$) compared to day 1, whereas the 5HT-D group did not show significant changes in swimming velocity [$F(5, 5.)=2.056$, $P=0.087$]. There were no inter-group differences in swimming velocity [$F(1, 21)=2408$, $P=0.136$] (Fig. 2C).

The number of crosses in the annulus central in each quadrant was compared using ANOVA and Tukey's test. Both groups had a greater number of crosses into the quadrant containing the platform than in the opposite quadrant ($P=0.001$ for both groups, Fig. 2D). The representative swimming route of one rat in each group is presented in Fig. 3.

3.3. Electroencephalogram

3.3.1. CA1

Intra-group comparisons of RP were made using an ANOVA test for block design considering three factors: Day (1–6), behavioural stage (basal, searching, platform) and frequency sub-band (low, high, maximum sub-band frequencies) and Tukey's *post-hoc* test. Intra-group comparisons of the vehicle group showed a significant interaction between stage and band factors [$F(4, 739)=149.703$, $P<0.001$]. For this group, paired comparisons between low and high sub-bands at each behavioural stage showed differences in the basal stage (higher low-frequency RP, $P<0.001$) and during the search stage (higher high-frequency RP, $P<0.001$), whereas no differences

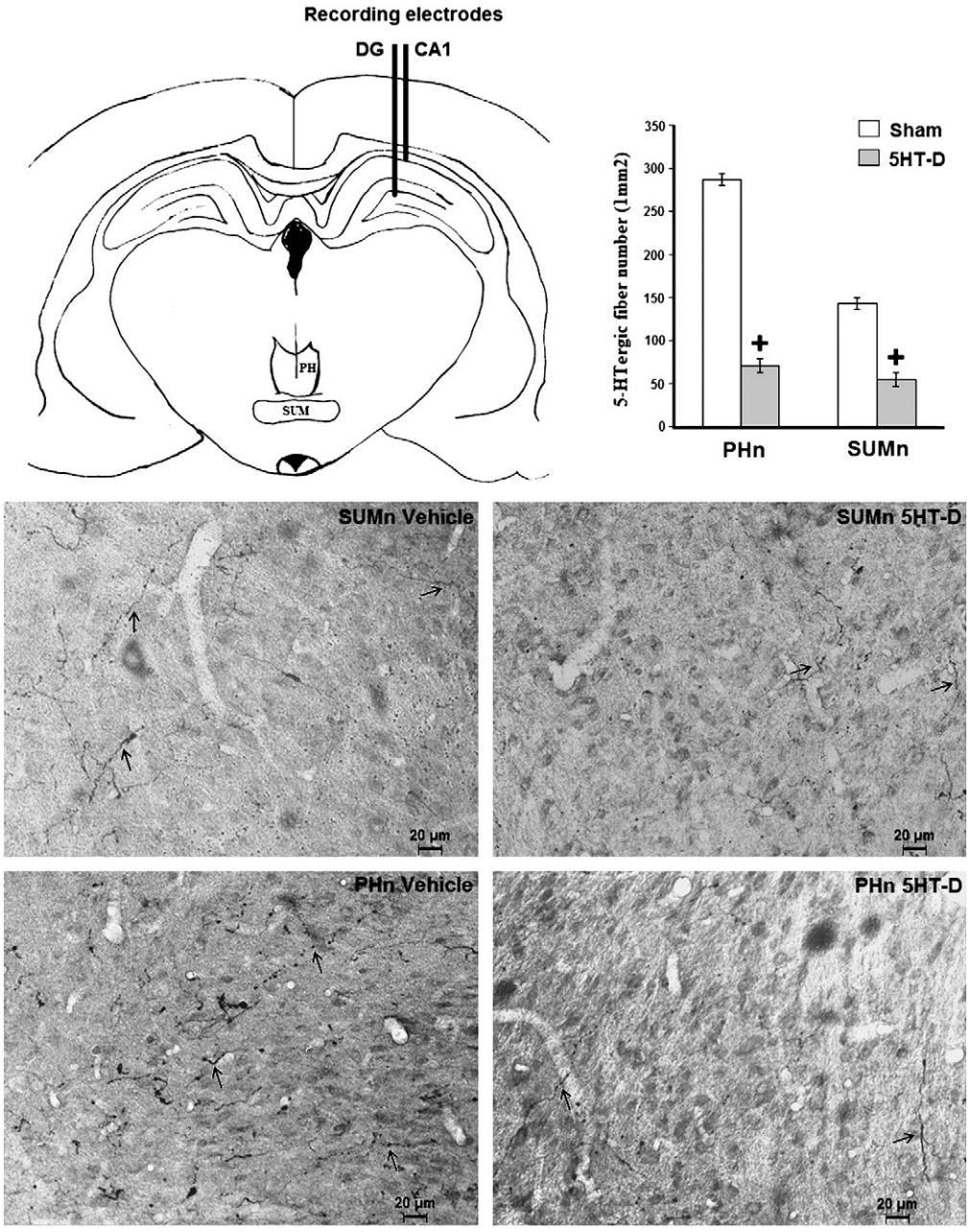

Fig. 1. Graph showing the number of 5-HT immunoreactive fibres in the SUMn and PHn in the two groups of rats. Mean ± S.E.M. *, $P<0.05$. A) Schematic representation of a coronal section including the SUMn and the PHn in the middle region, which was one of the three regions in which 5-HT immunoreactive fibres were counted. B) Microphotography of the corresponding slices of SUMn and PHn from the two groups of animals. Calibration, 250 µm; magnification, 20×.

were observed in the platform stage (Fig. 4A). The 5HT-D group also showed a significant interaction between behavioural stage and sub-band factors [$F(4, 530) = 28.519$, $P<0.001$]. For this group, the paired comparisons showed higher low-frequency RP during the basal ($P<0.001$) and platform ($P<0.001$) stages but were not significantly different between low and high sub-bands during the search for the platform ($P = 0.134$, Fig. 4A). The intra-group comparison revealed a significant interaction between day, stage and sub-band in the vehicle group [$F(20, 739) = 2.594$, $P<0.001$]. The paired comparisons showed a significantly higher high-frequency RP than a low-frequency RP from day two to six ($P \leq 0.001$) of training in the searching stage. Additionally, the high-frequency RP was higher on days three ($P = 0.001$) four ($P<0.001$), five ($P<0.001$) and six ($P = 0.003$) compared to the first training day, whereas the low-frequency RP was lower on days three ($P = 0.007$), four ($P = 0.016$) and five ($P = 0.025$) compared with the first day in the same stage (Fig. 4B). The intra-group comparison of the 5HT-D rats did not show significance interaction between day, stage and band [$F(20, 530) = 0.774$, $P = 0.745$] (Fig. 4B).

The inter-group comparisons (repeated measures ANOVA) considering either group, stage and frequency sub-band [$F(1, 224) = 2.329$, $P = 0.128$] or group, day, stage and frequency sub-band [$F(5, 1120) = 0.555$, $P = 0.735$] did not show significant differences. The main results are summarised in Table 1.

The correlation between both distance travelled and swimming velocity with the RP of the low and high-frequency sub-bands was obtained for each group (Pearson's correlation). The vehicle group showed a positive correlation between the RP of the low-frequency band and the distance travelled ($r = 0.262$, $P = 0.014$) and a negative correlation between high-frequency theta activity RP and the distance travelled ($r = -0.258$, $P = 0.015$). Additionally, a negative correlation was observed between swimming velocity and the RP of

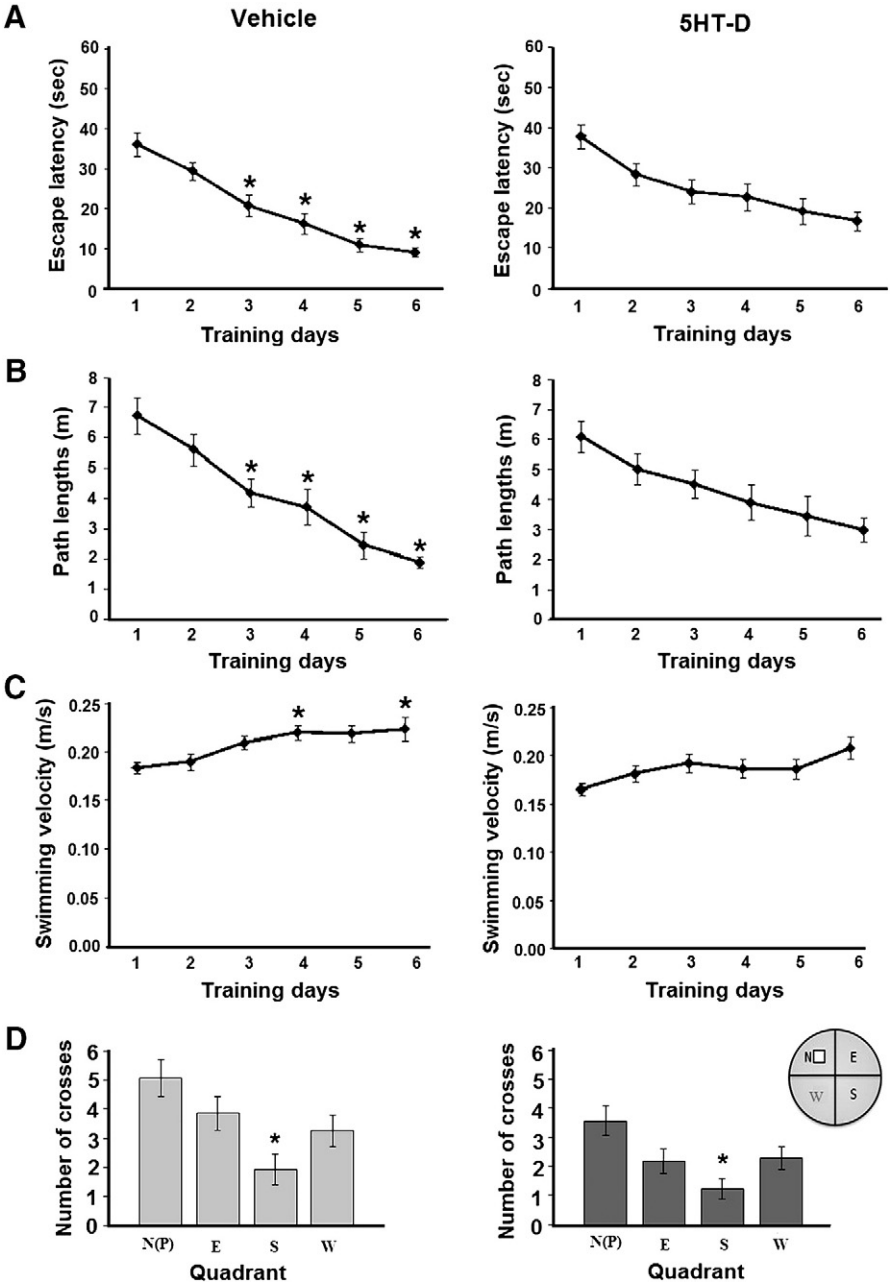

Fig. 2. Graph showing A) the escape latencies for the control (n = 15) and 5-HTD (n = 11) groups of rats on each day of training. Ordinate: latency (seconds); Abscissa: day of training. Median ± S.E. of the median. * First day versus subsequent days, P ≤ 0.005. B) Distance travelled by the two groups of rats on each day of training. Ordinate: paths length (metres); abscissa: day of training. Mean ± S.E.M. *First day versus subsequent days, P ≤ 0.005. C) Swimming velocity displayed by the two groups of rats for each day of training. Ordinate: velocity (m/s); abscissa: day of training. Mean ± S.E.M, *First day versus subsequent days, P < 0.05. D) Number of crosses into a central annulus in each quadrant: north (N), south (S), east (E), west (W), during the probe trial, Mean ± SEM. The platform was placed in the north quadrant (P) during the training. *Quadrant north versus all other quadrants, P = 0.001.

the low-frequency sub-band (r = −0.234, P = 0.028) and a positive correlation was observed between swimming velocity and the high-frequency sub-band RP (r = 0.249, P = 0.019). The 5HT-D group showed a significant positive correlation between distance travelled and the RP of the low-frequency sub-band (r = 0.275, P = 0.025), but no significant correlation between distance travelled and the RP of the high-frequency sub-band (r = −0.221, P = 0.074). Moreover, the 5HT-D group also showed a significant negative correlation between the swimming velocity and the RP of the low-frequency sub-band (r = −0.386, P = 0.001) and a positive correlation of swimming velocity with the RP of the high-frequency sub-band (r = 0.342, P = 0.005, Fig. 5).

3.3.2. Dentate gyrus

In the intra-group comparison of the factors, stage and frequency band (ANOVA for block design and Tukey's test) [F (4, 739) = 37.552, P < 0.001], the vehicle group showed higher low-frequency RP than high-frequency RP in the basal (P < 0.001) and platform (P < 00.001) behavioural stages, whereas in the searching stage, the high-frequency RP was higher than the low-frequency RP (P < 0.001, Fig. 6A). Additionally, the 5HT-D group showed a significant interaction between stage and frequency band [F (4, 530) = 27.743, P < 0.001]; the low-frequency RP was higher on the basal and platform stages (P < 0.001), but no difference was observed between the low- and high-frequency band RPs during the platform search (P = 0.197,

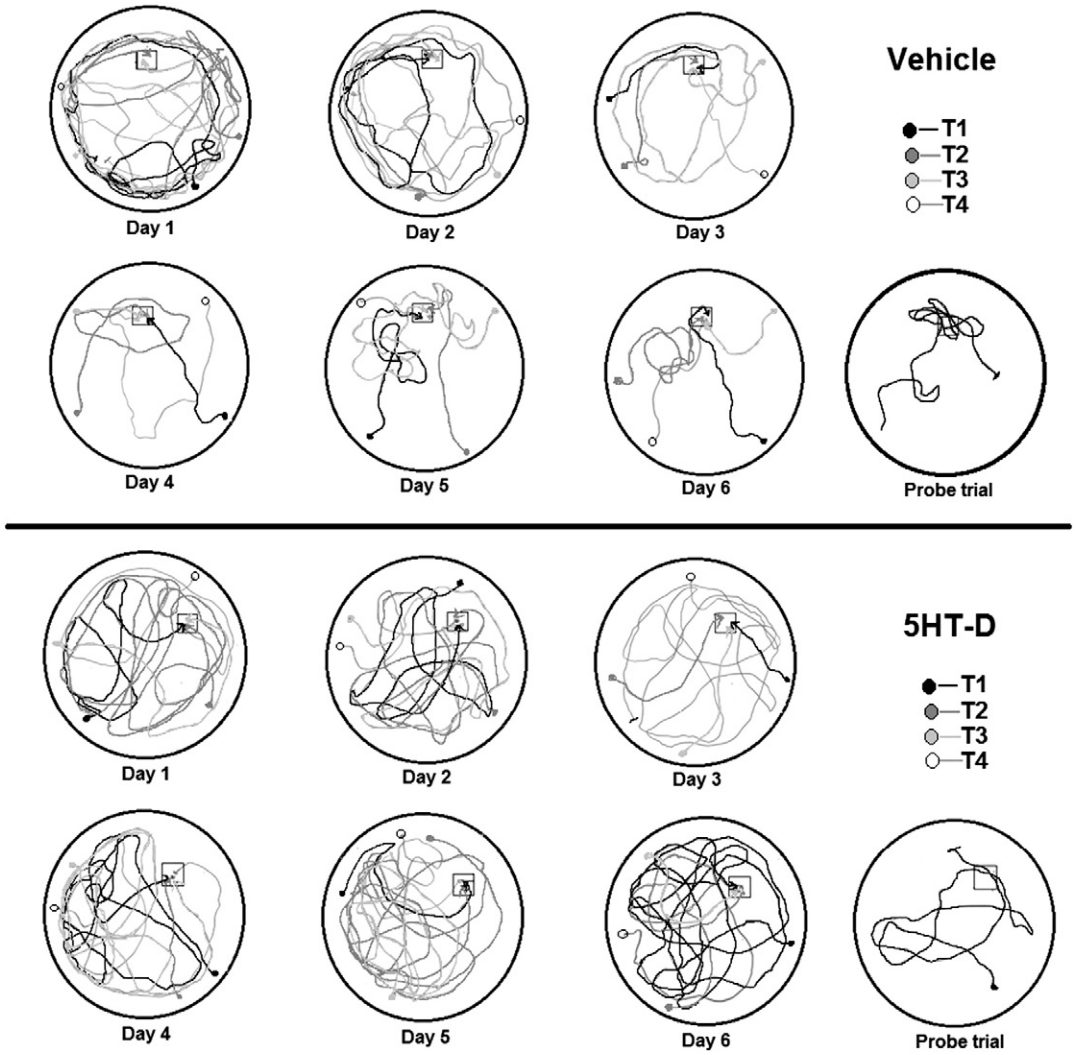

Fig. 3. Swimming routes displayed by one representative animal from each group on each training day (day 1–day 6) and during the probe trial. The four trials (T1–T4) are represented in different colours. The square indicates the platform position.

Fig. 6A). In the intra-group comparison, considering day, stage and frequency band, no significant interactions were observed between the factors for any group (Fig. 6B). The main results are summarised in Table 1.

The correlation between the RP of the low- and high-frequency sub-bands of the dentate gyrus and both the distance travelled and swimming velocity was obtained. The vehicle group did not show significant correlations between the distance and the low- ($r=0.053$, $P=0.624$) or high-frequency ($r=-0.064$, $P=0.549$) sub-bands. This group also did not show a significant correlation between swimming velocity and low- ($r=-0.090$, $P=0.406$) or high-frequency ($r=0.128$, $P=0.236$) sub-bands. The 5HT-D group displayed a significant positive correlation between distance travelled and low-frequency RP ($r=0.323$, $P=0.008$) and a negative correlation between distance and high-frequency RP ($r=-0.327$, $P=0.007$). Additionally, a significant negative correlation between swimming velocity and low-frequency RP ($r=-0.386$, $P=0.001$) and a significant positive correlation between swimming velocity with high-frequency RP ($r=0.247$, $P=0.041$) were observed (Fig. 7).

4. Discussion

It has widely been shown that the SUMn/PHn nucleus modulates the frequency of hippocampal theta activity in both anaesthetised and awake rats (Bland et al., 1994; Kirk and McNaughton, 1993; McNaughton et al., 2006; Oddie et al., 1994; Pan and McNaughton, 1997). Additionally, a relationship between SUMn functionality, theta modulation and cognitive functions has been shown (McNaughton et al., 2006; Pan and McNaughton, 1997). The serotonergic modulation of hippocampal theta activity has also been established (Vanderwolf et al., 1989; Vinogradova et al., 1999). However, how this system regulates hippocampal theta activity through each relay of the SAS remains unknown.

Substantial high-frequency theta activity in the CA1 region has been consistently shown in intact rats during Morris water maze test training. Briefly, dominant high-frequency theta activity was observed in the CA1 EEG recordings in rats trained in place-learning but not in rats trained in cue-learning (a non-hippocampal-dependent learning of stimulus–response associations evaluated using a visible platform) using the Morris water maze (Olvera-Cortes et al., 2002). In addition, an increase in the high-frequency theta activity throughout the training days and a correlation between distances and high-frequency theta activity (short distances were associated with major values and long distances with minor values of high-frequency theta activity) in the Morris maze were observed in rats trained in place-learning but not in rats trained in non-hippocampal-dependent learning (Olvera-Cortes et al., 2004). Thus, it has been hypothesised that the recruitment of high-frequency theta activity in the CA1 is necessary for efficient spatial learning (Gutierrez-Guzman et al., 2011; Olvera-Cortes et al., 2002, 2004, 2012).

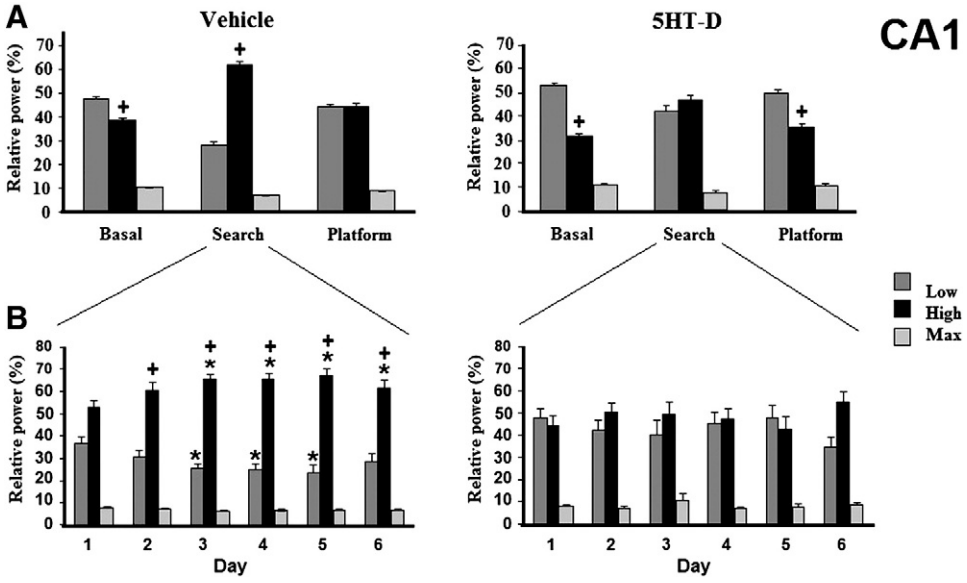

Fig. 4. A) Relative power (percent of the absolute power, %) of the CA1 theta activity recorded during each behavioural stage, on each training day, for the control (n = 15) and 5-HTD (n = 11) groups of rats. Ordinates: relative power; abscissas: behavioural stage (basal, searching and platform). B) Relative power (percent of the absolute power, %) of the CA1 theta activity recorded during the searching stage on each day of training, for the two groups of rats. Ordinates: relative power (%); Abscissas: day of training. Low-frequency (4–6.5 Hz, low), high frequency (7–9.5 Hz, high) and maximum frequency (10–12 Hz, max) sub-bands. Mean ± S.E.M. +, low-frequency versus high-frequency relative power; *, First day versus subsequent days of training, $P<0.001$.

The SUMn projections of the hippocampus occur predominantly on dentate gyrus granular cells and the CA2/CA3 field in the Ammon horn (Magloczky et al., 1994; Vertes, 1992). Also, the SUMn sends afferents, some of which are glutamatergic in nature, to cholinergic and GABAergic cells of the MS/vBDB (Leranth and Kiss, 1996). Although the PHn does not send direct projections to the hippocampus, it innervates the SUMn and septum (Vertes et al., 1995). The reversible inactivation of both SUMn and PHn to suppress all spontaneous- and induced- (sensorial or reticular stimulation) theta activity in anesthetised rats has been shown (Bland and Colom, 1993; Kirk and McNaughton, 1993; Oddie et al., 1994). However, there are differences in the results observed in freely moving rats, in which the influence of these nuclei on theta activity generation appeared to have only a modulatory role (McNaughton et al., 1995; Pan and McNaughton, 1997, 2002). The primary effect observed in the present work was related to alterations in frequency encoding, which is in line with the proposed role of these nuclei in theta modulation (Kirk and McNaughton, 1993). In the present work, substantial high-frequency theta activity in the searching stage was observed not only in the CA1 but also in the dentate gyrus in vehicle rats, but not in 5HT-D rats (see Figs. 4 and 6). Both groups of rats displayed the same swimming behaviour; however, only vehicle rats, which were efficient in solving the test, showed this high-frequency theta activity, adding support to the idea that these changes could be related to the processing of spatial information.

The learning-related changes in CA1 were more prominent than those in the dentate gyrus in vehicle rats and were in according with the previous findings and consisted of an increase in the proportion of high-frequency theta activity during the training days and a negative correlation between high-frequency theta activity and distance travelled (short trials were associated with higher high-frequency theta activity).

The 5HT-D rats showed neither an increase in theta proportion between days nor a significant correlation between high-frequency theta activity and distance travelled; all other correlations (low-frequency with distance and swimming velocity with low or high RP) remained unchanged (See Fig. 5). Thus, the high-frequency band more likely reflected the processing of spatial information.

In the dentate gyrus EEGs of the vehicle rats, the high-frequency theta activity was greater than the low-frequency band, but no increase in the high-frequency theta activity occurred through the training days. The differential learning-related changes on the CA1 and dentate theta activity observed in the vehicle rats could be related to the proposed dissociation of hippocampal sub-field function because the CA1 area has been related to the intermediate- and long-term consolidation of spatial information (Daumas et al., 2005; Kesner et al., 2004; Remondes and Schuman, 2004; Vago et al., 2007), whereas the dentate gyrus has been related to a network involved in pattern completion/separation, that participates in the discrimination between very similar spatial representations and in the encoding, but not the consolidation of acquired information (Jerman et al., 2006; Kesner, et al., 2004). The increased high-frequency theta activity during the searching stage was not observed in 5-HTD rats. Moreover, the 5HT-D rats showed significant correlations between the theta activity recorded in the dentate gyrus and the distance travelled, as well as between the swimming velocity and theta (low- and high-frequency) in this same region. These correlations were absent in the vehicle group in spite of their increased high-frequency theta activity during the searching stage. One possible interpretation is that, in absence of theta-related changes, the dentate and CA1 EEG of the 5HT-D rats reflected the theta component related to motor activity, which has been proposed to be atropine-sensitive and to have lower frequencies (Oddie and Bland, 1998; Oddie et al., 1997).

Unlike the results observed in the present study, it was reported that the SUMn minimally influenced the hippocampus during Morris water maze tasks; however, this result was observed in the performance of a one-day test in which the main influence of SUMn on CA1 hippocampal EEG was observed on the last training trials (Ruan et al., 2011). In the present work, we evaluated long-term place learning ability and observed marked deficiencies in the 5HT-D rats, which did not significantly reduce their distances travelled during the six days of training. A possible explanation of the difference between our results and previous reports could be that the SUMn/PHn participates in place learning ability via the modulation of concurrent theta activity, which occurs when consolidation of the information

Table 1
Relative power of the EEG recorded during the searching stage.

		CA1		Dentate gyrus	
Day	Group	Low frequency	High frequency	Low frequency	High frequency
1	Vehicle	36.93 ± 2.98	53.22 ± 3.06[a]	42.41 ± 2.77	45.42 ± 2.66
	5-HTD	46.97 ± 4.68	43.31 ± 4.72ns	45.97 ± 4.96	44.20 ± 4.96
2	Vehicle	30.48 ± 3.40	60.74 ± 3.59[a]	38.70 ± 4.29	50.96 ± 4.20
	5-HTD	41.50 ± 4.40	49.19 ± 4.49ns	42.25 ± 3.94	43.68 ± 4.04
3	Vehicle	25.70 ± 2.19	65.95 ± 2.23[a,b]	36.93 ± 3.96	52.48 ± 3.81
	5-HTD	39.53 ± 6.02	48.15 ± 6.24ns	41.06 ± 4.30	46.02 ± 5.42
4	Vehicle	25.25 ± 2.46	66.05 ± 2.57[a,b]	39.54 ± 4.91	50.19 ± 4.56
	5-HTD	44.15 ± 5.16	46.42 ± 5.21ns	38.77 ± 4.58	49.19 ± 4.51
5	Vehicle	23.74 ± 3.39	67.35 ± 3.24[a,b]	34.39 ± 5.31	54.15 ± 4.78
	5-HTD	47.13 ± 5.54	42.00 ± 5.62ns	40.16 ± 5.42	49.58 ± 5.45
6	Vehicle	28.95 ± 3.42	61.67 ± 3.49[a,b]	39.59 ± 4.56	50.08 ± 4.70
	5-HTD	34.26 ± 4.26	54.51 ± 4.71ns	42.93 ± 4.20	45.81 ± 5.04
1–6	Vehicle	28.51 ± 1.29	62.50 ± 1.33[a]	38.59 ± 1.75	50.55 ± 1.68[a]
	5-HTD	42.26 ± 2.07	47.26 ± 2.10ns	41.85 ± 1.82	46.41 ± 1.95ns

[a] Difference between low and high frequency sub-bands.
[b] Differences between the first day of training and the other training days. $P<0.05$.

in long-term memory takes place. This view is in accordance with the results reported by Shahidi et al. (2004), in which the reversible inactivation of SUMn produced impaired consolidation of reference memory evaluated by the Morris water maze test. Thus, a differential role would exist for SUMn/PHn in modulation of learning in terms of short-term memory or long-term consolidation.

Previously, we observed that significant serotonin depletion, specifically in the hippocampus, produced facilitated place learning, which was associated with the earlier expression of predominant high-frequency theta activity throughout the training days (Gutierrez-Guzman et al., 2011). In the present work, the SUMn/PHn serotonin depletion produced an adverse effect in the spatial learning associated with a lack of learning-related predominant high-frequency theta activity in the hippocampus. It has been reported that serotonin exerts both inhibitory and excitatory influences on hippocampal GABAergic interneurons through 5-HT1A (Segal, 1990) and 5-HT3 receptors (Staubli and Xu, 1995), respectively. Furthermore, direct inhibition of principal cells via 5-HT1A receptors and indirect inhibition via excitation of GABAergic interneurons have been described; in the dentate gyrus (Kasamo et al., 2001; Piguet and Galvan, 1994). Serotonin, however, reached the SUMn and PHn, where it has been proposed to exert inhibitory influences on acetylcholine release and, through the activation of (calbindin positive) GABAergic neurons, similarly to its influence on the hippocampus (Jeltsch-David et al., 2008; Miettinen and Freund, 1992a, 1992b; Vertes, 2005). Thus, a reduction of the inhibitory tone on these two nuclei by 5-HT deafferentation would have induced increased cholinergic and glutamatergic neurotransmission in the septum and hippocampus, resulting in a deficiency of finely-tuned theta activity in relation to information processing, thus producing a continuous, inflexible, rhythmic oscillation incompatible with information transfer from the CA1 to the neocortex that is required for the efficient consolidation of spatial memory (Treves and Rolls, 1994). Whereas, in our previous work the direct elimination of the serotonin inhibitory milieu of the hippocampus apparently facilitated the engagement of the neurons into high-frequency theta activity, possibly facilitating the influence of the intact ASS on the serotonin-depleted hippocampal neurons.

It is important to note that, as in our previous work, we did not observe continuous hippocampal theta activity after the SUMn/PHn serotonin depletion, nor did we observe changes in the theta activity under basal conditions, indeed we only observed changes under conditions of the processing of information by the hippocampus. Thus, the serotonin appeared to be modulating the theta activity in a more complex manner than simply acting as a general inhibitor in each relay of the ASS and could be participating at least in the SUMn/PHn relay of

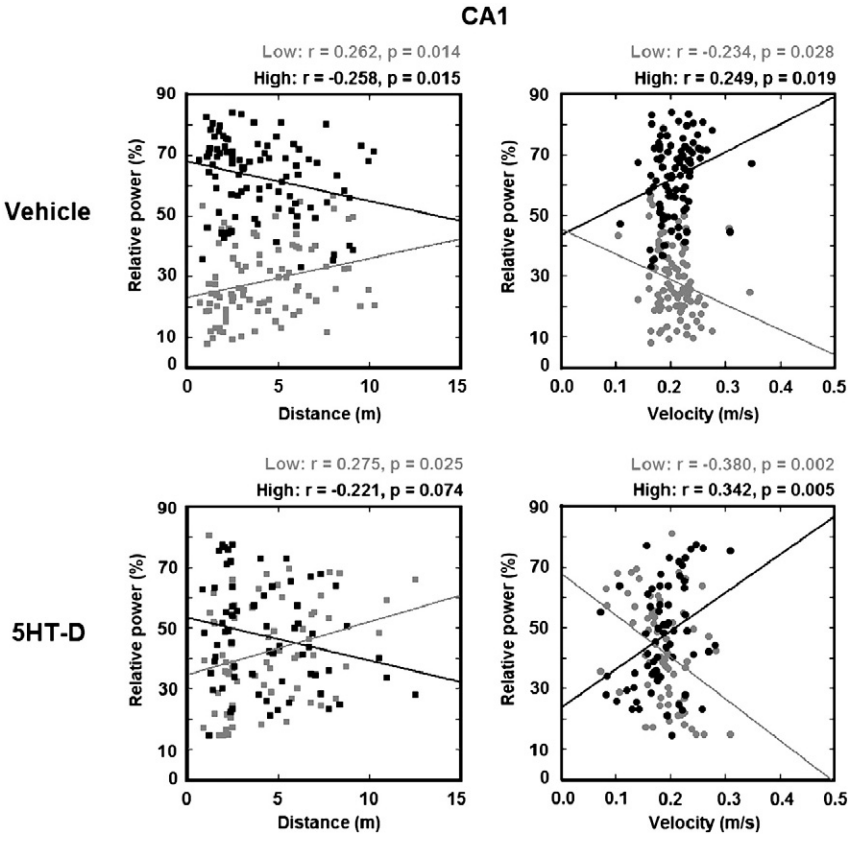

Fig. 5. CA1 theta activity: Pearson correlation between relative power of the low-frequency sub-band and distance travelled (grey squares) or swimming velocity (grey dots) and the Pearson correlation between high-frequency sub-band relative power and distance travelled (black squares) or swimming velocity (black dots) on each day of training of the two groups of rats. Ordinates: relative power (%); abscissas: distance travelled (m) or swimming velocity (m/s).

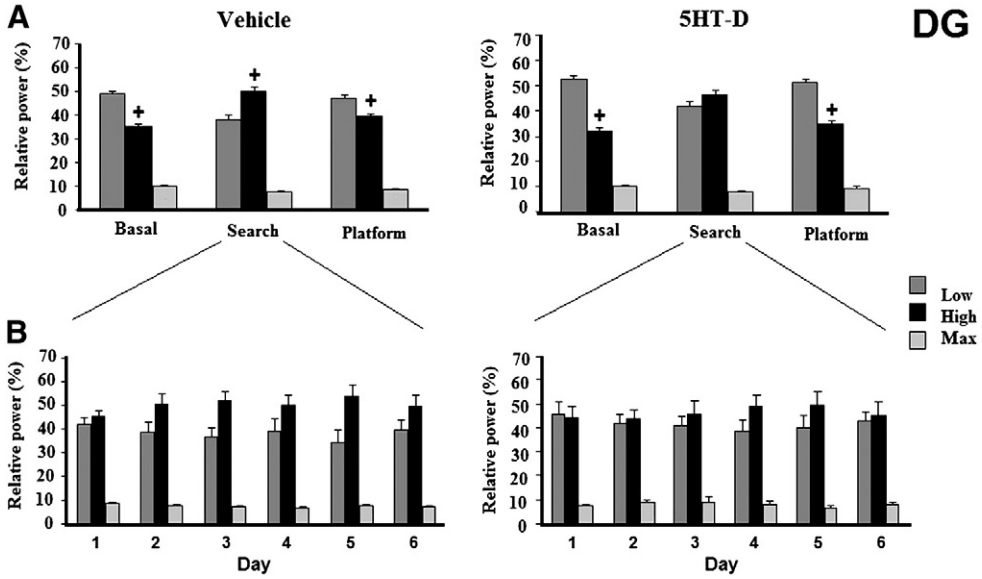

Fig. 6. A) Relative power (percent of the absolute power, %) of the dentate gyrus theta activity recorded during each behavioural stage on each training day for the control (n = 15) and 5-HTD (n = 11) groups of rats. Ordinates: relative power; abscissas: behavioural stage (basal, searching and platform). B) Relative power (percent of the absolute power, %) of the dentate gyrus theta activity recorded during the searching stage on each day of training for the two groups of rats. Ordinates: relative power (%); abscissas: day of training. low-frequency (4–6.5 Hz, low), high-frequency (7–9.5 Hz, high) and maximum frequency (10–12 Hz, max) sub-bands. Mean ± S.E.M. +, low-frequency versus high-frequency relative power; *, First day versus subsequent days of training, $P<0.001$.

the ASS, in the fine learning-related frequency modulation of the hippocampal theta activity. However, the information about the precise role of serotonin in the modulation of SUMn/PHn physiology is scarce; hence, the inference of mechanisms involved in its regulation of theta activity would be highly speculative. Moreover, the precise contribution of each nucleus (SUMn or PHn) to the electrophysiological and behavioural modifications observed in the 5HT-D rats studied in the present work needed to be elucidated.

In conclusion, the present results indicated that 5-HT can regulate the hippocampal theta activity acting on the SUMn/PHn relay of the

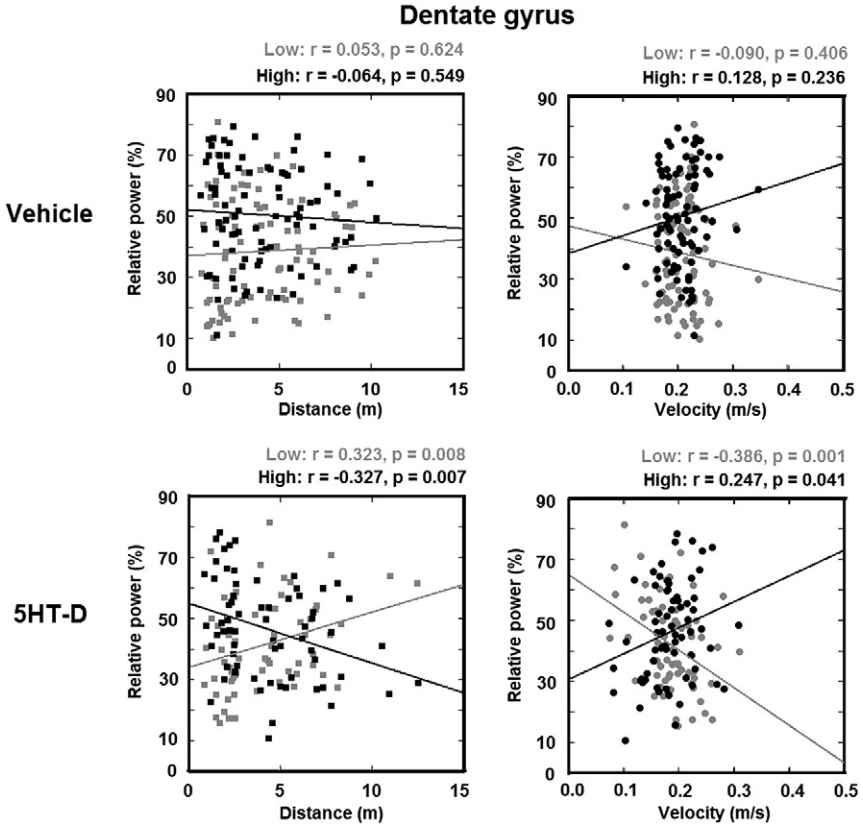

Fig. 7. Dentate gyrus theta activity: Pearson correlation between relative power of the low-frequency sub-band and distance travelled (grey squares) or swimming velocity (grey dots), and Pearson correlation between high-frequency sub-band relative power and distance travelled (black squares) or swimming velocity (black dots) on each day of training of the two groups of rats. Ordinates: relative power (%); abscissas: distance travelled (m) or swimming velocity (m/s).

SAS and that the influence of 5-HT on these nuclei is required for the display of learning-related changes in hippocampal theta activity underlying the resolution of the Morris water maze task and for successful behavioural performance. Thus, we found new evidence that 5-HT regulated the theta activity and the accuracy of hippocampal-dependent learning.

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