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TITOLO DELLA TESI DI DOTTORATO

"Role of dorsal and ventral hippocampus in working memory load capacity "

S.S.D. BIO16

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1.Abstract

The hippocampus has been traditionally associated to spatial long-term memory (LTM). It is believed that the hippocampus has a limited role in working memory (WM). Nevertheless, recent evidence suggested that it is involved in WM in high memory load (HML) conditions. The WM load is the number of elements retained in memory for a short time interval. This number of elements is limited and it is called working memory capacity (WMC). The aim of this work is to study the role of the hippocampus in WMC in CD1 mice. Anatomic studies suggested, however, that the hippocampus is subdivided into distinct dorsal and ventral portions. To study the role of the dorsal and ventral hippocampus in WMC in CD1 mice we used a neurotoxic selective dorsal and ventral hippocampal lesion approach. We tested control and lesioned mice in a WMC version of the radial maze task using a confinement procedure to force the animals to rely on allocentric spatial information. Both lesioned groups showed impaired spatial WMC. Removal of the confinement procedure favored in control mice the use of a sequential egocentric strategy, which lowered the number of errors by lowering the memory load. Dorsal hippocampus lesioned mice shifted to the sequential strategy as well as control mice, and showed impaired performance only with the highest memory load. In contrast, the ventral lesioned group showed a major deficit in the acquisition of the sequential strategy, and a consequent impaired WM performance. Then, when these same mice have been tested in a WMC task for objects, only the dorsal group showed the impairment. Finally, we tested both control and lesioned mice in a massive protocol of the Morris water maze task, the classical hippocampus - dependent spatial LTM task and both lesioned groups were impaired. These data suggest that both the dorsal and the ventral hippocampus are involved in WMC, as well as in LTM, for spatial information. The ventral hippocampus is more involved in mediating the acquisition of egocentric strategies to solve a spatial task. In contrast, only the dorsal part regulates WMC for objects. Therefore, this study provides an important contribution to the role of the hippocampus subregions along its septo-temporal axis in WMC.

1.1.Sommario

L’ippocampo è stato da sempre associato alla memoria spaziale a lungo termine (LTM) mentre il suo ruolo nella memoria di lavoro (WM) si credeva limitato. Recenti studi hanno però suggerito un coinvolgimento dell’ippocampo nella WM in condizioni di alto carico di memoria. La capacità di memoria è definita, infatti, come il numero di informazioni che un soggetto può ricordare in un breve intervallo di tempo (WMC). Lo scopo di questo lavoro è valutare il ruolo

dell'ippocampo nella WM in topi CD1. Studi anatomici suggeriscono che l'ippocampo è suddiviso in due regioni: dorsale e ventrale. Per studiare il ruolo di entrambe le regioni ippocampali, sono state effettuate lesioni selettive usando un agonista dei recettori glutammatergici NMDA. Successivamente entrambi i gruppi lesionati sono stati testati in una versione modificata del labirinto radiale a otto bracci che permette di valutare la WMC spaziale cambiando il numero di bracci aperti fra i trials e i giorni e usando una procedura di confinamento per "costringere" gli animali a fare riferimento alle informazioni spaziali allocentriche. Il passaggio dalla procedura di confinamento a quella di non confinamento favoriva lo sviluppo della strategia sequenziale nel gruppo controllo con una conseguente riduzione del numero di errori dovuta alla riduzione del carico di memoria. Il gruppo lesionato nell'ippocampo dorsale, come il gruppo controllo, nel passaggio dalla procedura di confinamento a quella di non confinamento, mostrava anch'esso un utilizzo della strategia sequenziale, dimostrando un aumento del numero di errori in condizioni di alto carico di memoria. In contrasto il gruppo lesionato nell'ippocampo ventrale mostrava un deficit nell'acquisizione della strategia sequenziale. Quando testati nel test di capacità di memoria ad oggetto con 6 differenti oggetti, solo i topi lesionati nell'ippocampo dorsale presentavano un difetto. In ultimo, entrambi i gruppi sperimentali sono stati testati nel classico test ippocampo-dipendente usato per valutare la memoria a lungo termine: il labirinto acqua di Morris in cui entrambi mostravano un deficit. Questi dati suggeriscono che sia l'ippocampo dorsale che quello ventrale sono coinvolti nella WMC, come nella memoria a lungo termine. L'ippocampo ventrale è più coinvolto nel mediare l'acquisizione di strategie egocentriche per risolvere un compito spaziale, in contrasto, l'ippocampo dorsale regola la WMC ad oggetto. Questo studio fornisce un importante contributo nel definire il ruolo delle subregioni ippocampali nella WMC.

2. Introduction

2.1. Anatomy of the hippocampal formation and organization of its intrinsic and extrinsic connections

The hippocampus proper is a part of an extended anatomic formation: hippocampal formation which is divided in four regions: the dentate gyrus, the hippocampus proper (which is divided in three subfields: Cornus Ammonis 3, Cornus Ammonis 2 and Cornus Ammonis 1 or CA3, CA2 and CA1), the subicular complex (which can be divided in three subdivision: subiculum, presubiculum and parasbiculum) and the enthorinal cortex which in rodents is divided in medial and lateral subdivisions. The neural population of CA1 consists principally of small pyramidal

neurons, while CA2 and CA3 region are characterized by a population of big pyramidal neurons. The basic knowledge on connections of the hippocampus comes from the classical Golgi studies of Ramón y Cajal and Lorente de Nò and from degeneration studies performed by (Blackstad 1956, Blackstad, Brink et al. 1970). The dentate gyrus receives projections from the enthorinal cortex through the perforant pathway, and in turn, granule cells of the dentate gyrus project through the mossy fibers to the CA3 region, which gives rise to connection in the same CA3, and to the CA1 region through the Schaffer's collaterals. Perforant path axons make excitatory synapsis with dendrites of granule cells; these cells, through the mossy fiber, project to dendrites of CA3, which, in turn, project to the ipsilateral CA1 pyramidal cells through Schaffer's collateral and to contralateral CA3 and CA1 pyramidal cells through commissural connections. Except for the sequential trisynaptic circuit, there are dense associative networks which interconnect CA3 cells on the same side. CA3 cells receive input also from layer II of the enthorinal cortex. The distal apical dendrites of CA1 pyramidal neurons also receive a direct input from layer III cells of the enthorinal cortex. The three major subfields of the hippocampus have a lamilar organization in which cell bodies are packed in a C - shape arrangement (Neves, Cooke et al. 2008) (**Figure 2.1.)**.

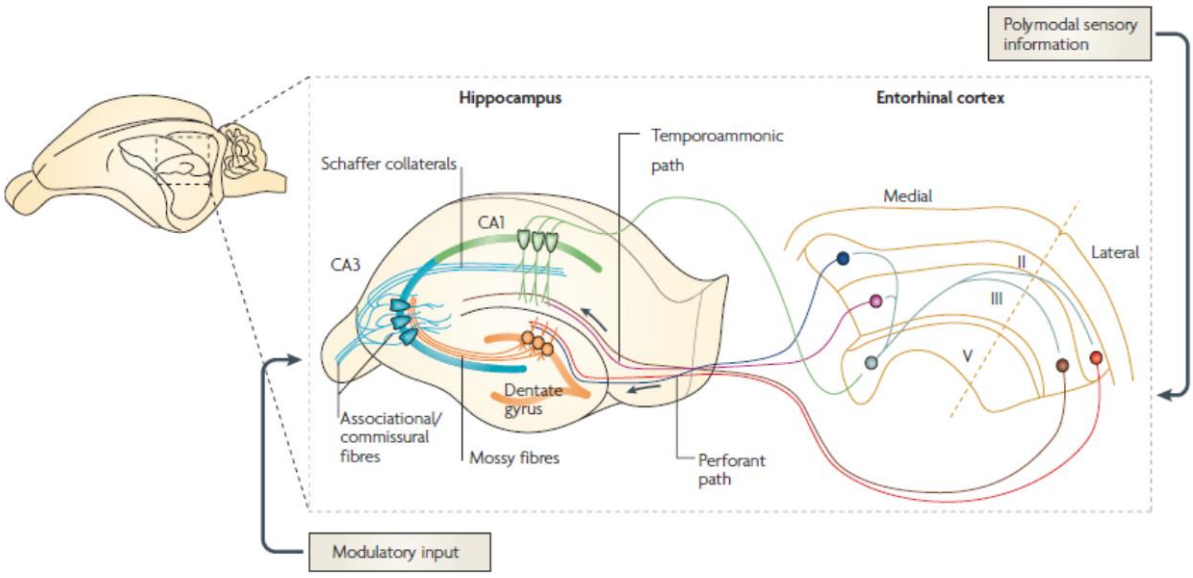


Fig. 2.1. Basic anatomy of the hippocampus (Neves, Cooke et al. 2008).

2.1.2. Anatomic distinction of dorsal and ventral region of the hippocampus formation

Ramón y Cajal in 1901 and Lorente de Nó in 1934 were the first to study the basic cytoarchitectonic structure of the hippocampus. Their work showed the distinct morphological properties of small pyramidal neurons in CA1 (region superior of Cajal), large pyramidal neurons of CA3 (region inferior of Cajal, in which mossy fiber are present) and CA2. Cajal was the first to observe a difference in the hippocampus across its dorsal to ventral axis. He distinguished two perforant paths from the enthorinal cortex namely "superior" and "inferior" that project respectively to dorsal and ventral hippocampus; Lorente de Nó also divided the "ammonic system" into three main segments along its longitudinal axis according to their different input.

2.1.3. Neural connectivity of the dorsal hippocampus

Dorsal CA1 contains place cells (Jung, Wiener et al. 1994, Muller, Stead et al. 1996), which code spatial location and send excitatory projections to the dorsal presubiculum and para-subiculum (Swanson and Cowan 1975, van Groen and Wyss 1990, Witter and Groenewegen 1990). The dorsal part of the subicular complex contains "head direction cells" which code for head direction in the space. Dorsal CA1 and the dorsal region of the subiculum complex send their projections to the retrosplenial and anterior cingulated cortices (van Groen and Wyss 1990, Muller, Stead et al. 1996) which are important for cognitive processes and to encode visuo - spatial information. The dorsal region of the subiculum sends projection to medial and lateral mammillary nuclei and to anterior thalamic complex (Swanson and Cowan 1975, Kishi, Tsumori et al. 2000, Ishizuka 2001). These two structures contain neurons related to the navigation. In turn, these structures send their projection back to the dorsal hippocampus and retrosplenial cortex (Risold, Thompson et al. 1997). Dorsal CA1 and CA3 regions send projection to the lateral septum, which in turn is connected to the medial septal complex and supramammillary nucleus (Risold and Swanson 1996). These two structures generate and control theta rhythm during voluntary locomotion. The dorsal subiculum and lateral band of the medial and lateral enthorinal cortex send their projection to the rostro – lateral part of the nucleus accumbens and caudate putamen, which, in turn, project to the ventral tegmental area and to the substantia nigra pars reticulata. These structures are involved in locomotion and well-defined movement. Dorsal

hippocampus - subicular complex made a network with the retrosplenial and anterior cingulate cortex and this complex have a critical role in cognitive processes as learning, memory, navigation and exploration (**Figure 2.1.2**.).

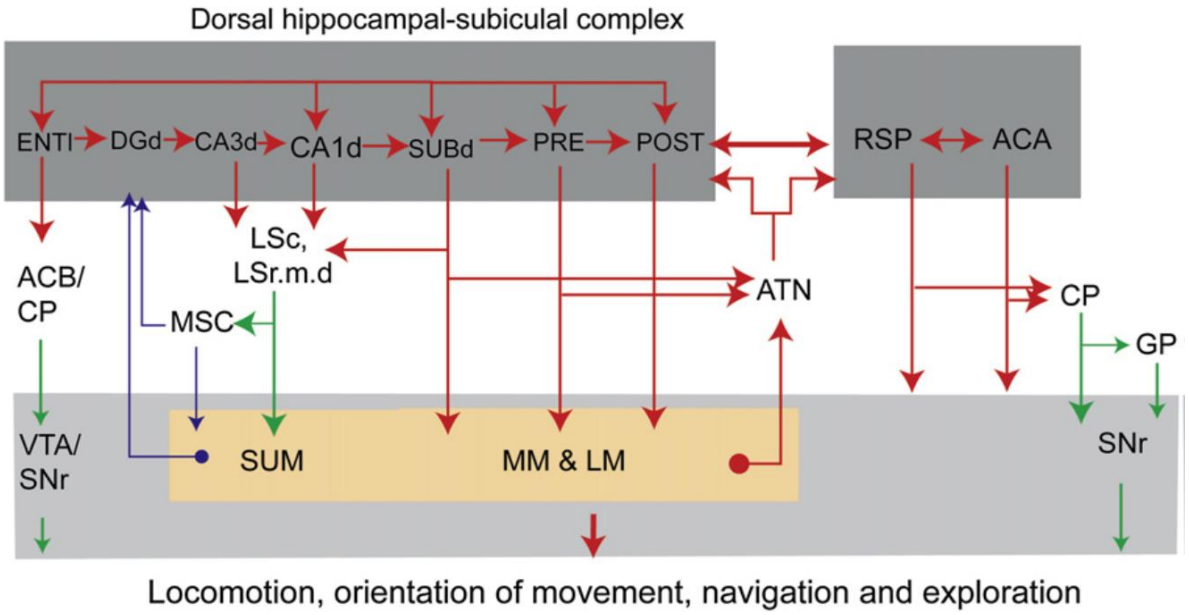


Fig.2.1.2. Schematic representation of the connectivity of the dorsal hippocampus. Abbreviations: ACA, anterior cingulated area; ACB, nucleus accumbens; ATN, anterior thalamic complex; CP, caudoputamen; DGd, dorsal domain of the dentate gyrus; ENTl, the caudolateral band of the entorhinal cortex; GP, globus pallidus; LM, lateral mammilary nucleus; LSc, the caudal part of the lateral septal nucleus; MM,medial mammilary nucleus; MSC, medial septal complex; PRE, presubiculum; POST, postsubiculum; RSP, retrosplenial cortex; SNr, reticular part of the substantial nigra. SUBd, dorsal subiculum; SUM, supramammillary nucleus; VTA, ventral tegmental area (Fanselow and Dong 2010).

2.1.4. Neural connectivity of the ventral hippocampus

Ventral CA1 projects to the olfactory bulb and to other primary olfactory cortices (anterior olfactory nucleus, piriform and endopiriform nucleus) (Cenquizca and Swanson 2007). These connections could have a role in depression-like symptoms following the damage of the loss of the olfactory bulb. In addition, ventral CA1 have bidirectional projection with amygdlar nuclei. The ventral hippocampus-subiculum-amygdalar complex and medial prefrontal cortex send projection to the periventricular and medial zone of the hypothalamus, which are involved in neuroendocrine, autonomic and somatic motor activity. Ventral CA1-subiculum-amygdalar nuclei are involved in the control of neuroendocrine activity with their strong projection to the ventral part of the lateral septum and to the bed nuclei of the stria terminalis (Canteras, Simerly et al. 1992). In addition, both ventral CA1 and subiculum send projections to the central

amygdalar nucleus (Kishi, Tsumori et al. 2006, Cenquizca and Swanson 2007) that play an important role in fear. Finally, ventral CA1- subiculum and lateral and medial enthorinal cortex send projections to the nucleus accumbens shell which has an important role in reward and feeding behaviour. Ventral hippocampus has an important role in regulating emotional states (**Figure 2.1.3)**.

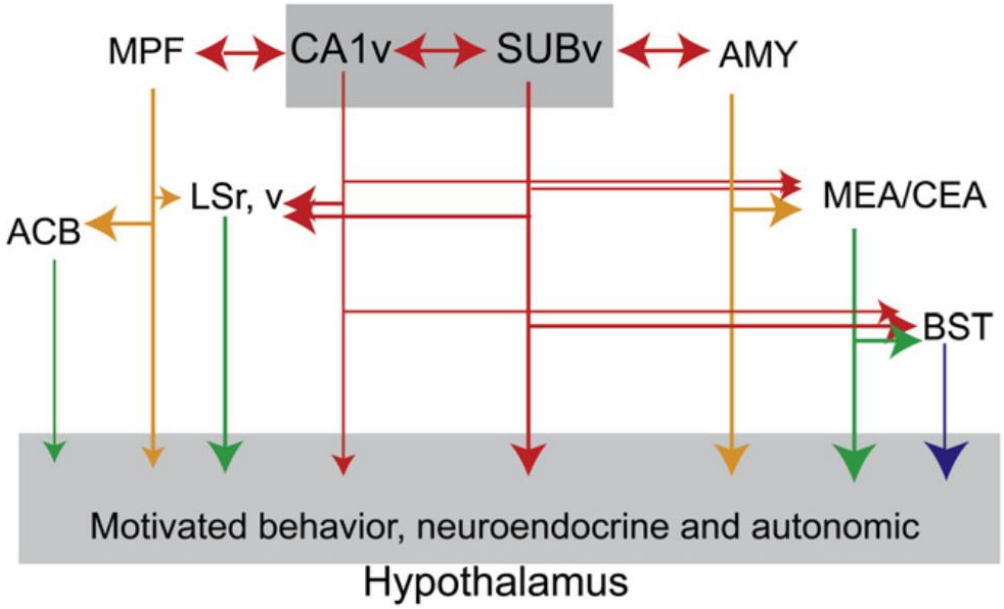


Fig.2.1.3. Schematic representation of the connectivity of the ventral hippocampus. Abbreviations: ACB, nucleus accumbens; AMY, cortical-like amygdalar areas(nuclei); BST, bed nuclei of the stria terminalis; CEA, central amygdalar nucleus; LSr, v, the rostral and ventral parts of the lateral septal nucleus; MEA, medial amygdalar nucleus; MPF, medial prefrontal cortex; SUBv, the ventral subiculum (Fanselow and Dong 2010).

2.2. Learning and memory

Memory was defined as the current knowledge about something that was presented previously (Rubinstein, 1988). In the past, the dominant view considered memory as an unitary process. Today, memory is considered to take multiple forms and types of brain functions. Learning is defined as the change in the behavior based on the experience, while memory is the retention and storage of information acquired during learning. According to Atkinson and Shiffrin, 1968, memory is composed of three interconnected memory stores, (multi store model of memory). Information is initially stored in sensory memory (SM), for only few seconds. This time interval allow us to decide what kind of information are important to transfer to WM. In the WM the information processing is continued. WM can held only about 7 bits of information (for example words, number, letters) for about 30 seconds unless we continue to maintain through repetition

(Miller 1956, Baddeley, Logie et al. 1986, Greene and Crowder 1986). The repetition process is called rehearsal and it is important, in first instance, to maintain information in WM as long as we repeat and, in second instance, to transfer information at the third and finally store, LTM. The process by which items are transferred from short - term memory (STM) to LTM is the synaptic consolidation. During the first minutes or hours after the acquisition, the memory trace is encoded within synapse, becoming resistant (although not immune) to interference from outside sources (Dudai 2002, Dudai 2003).

2.2.1. Working memory

Atkinson's and Shiffrin's multi store model of memory had a dominant role in the 1960's. But their model was discussed especially by Baddeley and Hitch in 1974, who would to investigate the link between STM and LTM, According to Atkinson and Shiffrin, retaining information in STM guarantee transfer to LTM, but Craik and Lockhart in 1972, demonstrated that the nature of processing of the information is important, with a deeper and more elaborated processing leading to better learning. A second point was that short-term processing was essential to store information in LTM, but there are inconsistences with neuropsychological cases. A third point was that Atkinson and Shiffrin considered STM as WM playing a role in cognition, so patients with impairment in STM could have intellectual deficits, even if there was a patient, who was for example an efficient secretary. These evidence led to Baddeley and Hitch in 1974 to formulate their theory on WM, in which WM refers to a brain system that provides temporary storage and manipulation of information necessary for complex cognitive tasks as language comprehension, learning and reasoning. WM is composed of three different components:

- The central executive , which is an attentional - controlling system, and it is important for tasks like playing chess and it is particularly susceptible to effects of Alzheimer's disease and its "slaves":

1. The visuo spatial sketch pad which manipulates visual images
2. The phonological loop, which stores and reharses speech-based information and it is necessary for the acquisition of both native and second-language vocabulary (**Figure 2.2.1.**).

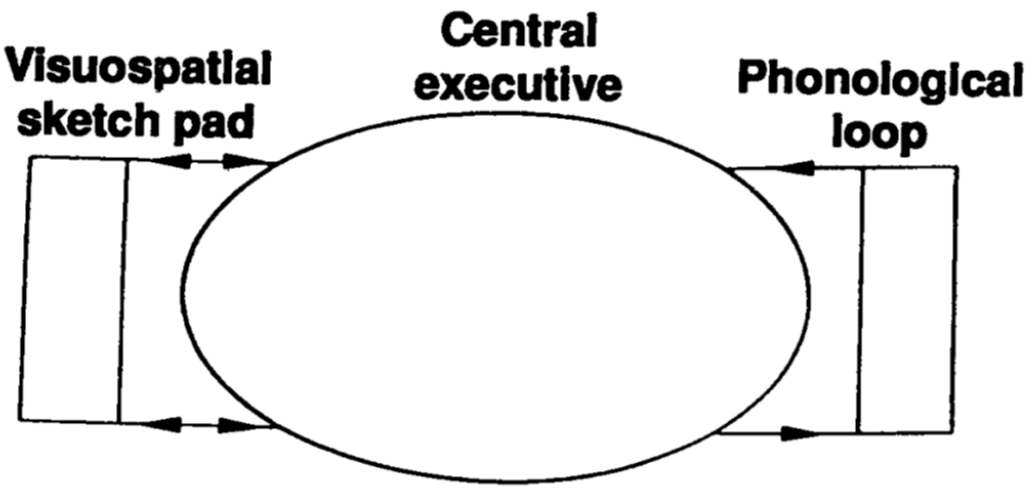


Fig.2.2.1. Shematic representation of the Baddeley and Hitch working memory model (Baddeley 1992).

How is defined WM in rodents? The first use of the term WM to rodents comes from studies of Olton and Samuelson in 1976 who devised a classical task to assess WM in rodents, the radial arm maze and formulated their hypothesis about WM in rodents: WM is the memory that allows the animal to remember an arm that was already visited. It is defined as a STM for an object, stimulus or location that is used within a testing session, but not typically between sessions, differently from reference memory (RM) which is acquired with repeated training and would last from days to months. RM is defined as a memory for the "rules" of a given task, for example, press a lever in order to obtain food pellet or find an hidden platform in the water maze. WM is a delay-dependent representation of stimuli that are used to guide behaviour within a task. For Olton and Samuelson WM was different from RM because it uses flexible stimulus - response associations and it is sensitive to interference. However, it could be difficult to distinguish between STM and WM in rodents. WM is a STM that once used, should be forgotten or ignored. It is useful for rodents, for example, to remember which arm they have visited. Finally, a key concept in the WM is the WM capacity or span, which is the amount of information that can be retained in memory for a short time interval. George Miller in 1956 suggested that in humans WM capacity (WMC) is 7± 2 number of items. WM impairments are found in schizophrenia and schizophrenia- spectrum patients, who present impairment in spatial, verbal/auditory, object and haptic WM (Park, 2014). One of the most characterised animal model of schizophrenia is the neonatal ventral hippocampal lesion model first developed by Lipska and colleagues (Lipska, 1993, Tseng, 2009).

2.2.2. WM and hippocampus: new perspectives

Historically, WM has been associated to cortical regions such as prefrontal cortex, perirhinal cortex, with no involvement of the hippocampus. Indeed, hippocampus is usually linked to LTM but there are evidence for which is required in WM maintenance of novel information (Ranganath and D'Esposito 2001, Axmacher, Mormann et al. 2007, Axmacher, Henseler et al. 2010, Fuentemilla, Penny et al. 2010, Poch, Fuentemilla et al. 2011). It has been shown that after a presentation of a to - be - remembered stimulus, hippocampus neural representation is elevated in medial temporal lobe (Fuentemilla, Penny et al. 2010, Poch, Fuentemilla et al. 2011). Studies from Ben - Yakov and Dudai in 2011 (Ben-Yakov and Dudai 2011) were very important to investigate on the relation between hippocampus and WM. They performed experiments with functional Magnetic Resonance Imaging (fMRI) to identify which brain regions were activated in patients after the presentation of complex stimuli, as movie clips, and correlate them with the subsequent recall. They reported a bilateral activation of the hippocampus after the stimulus presentation. In another experiment, they investigate whether the activation of the hippocampus is dependent on the stimulus duration or simply on the offset of the stimulus. In order to study this, they presented movies of different lengths, and they observed that the hippocampal response was related more to the offset of the stimulus rather than on the duration. In an another experiment, they studied the activation caused by movie clips of different lengths but with the same beginning and they reported that the bilateral activation of the hippocampus and caudate nucleus is time locked to the offset of the presentation of the stimulus and it is predictive for subsequent recall. This post - stimulus activity reflects the process of binding experience into cohesive units and registering into memory.

2.3. Tasks to assess WM in rodents

2.3.1. Delayed matching /non matching to sample with objects, odours

Delayed non- matching to sample (DNMS) task require a rodent to remember a stimulus over a delay in which the stimulus is not presented. After the delay period, the rodent is presented with the to-be-remembered stimulus and a novel stimulus, and the rodent is reinforced whether it chooses the novel stimulus. In the delayed matching to sample (DMS) tasks, rodents are rewarded to select the to -be-remembered stimulus. An example of DNMS task comes from the study of Aggleton et al in 1985, where rats are trained on a Y-shaped maze, where boxes containing different stimuli are placed at the end of each maze arm. On a given trial two boxes

are identical, and after a delay of 20 sec, one box is replaced with a novel box. Rats are rewarded to choose the novel box. An example of DNMS task with objects can be find in the study of Rothblat and Hayes (Rothblat and Hayes 1987). They used a version of DNMS task with trial unique stimuli. In their task, rats were presented with a sample object at the end of a straight runway. The rat had to displace the object to obtain food reward. After a 10 sec of delay period, rat again ran down the runway and was presented both the sample object and a novel object. In this case, reward was provided if rat displace the novel object. An example of DNMS with odours could be find in Dudchenko et al. in 2000 (Dudchenko, Wood et al. 2000) in which rats were presented with a cup of sand scented with a spice. Rats had to dig in the sand to obtain a food reward, after a short delay, the same cup of sand scented with the first odour was presented with a new cup of sand scented with another spice, rats were require to dig in the second cup to obtain a reward. After another delay, the rat was presented with three cups of sand, two of which were already presented, and a third novel cup scented with a different spice, even in this case the rat was rewarded to dig in the cup with the novel scent. In this way WMC could be assessed **(Figure 2.3.1.)**.

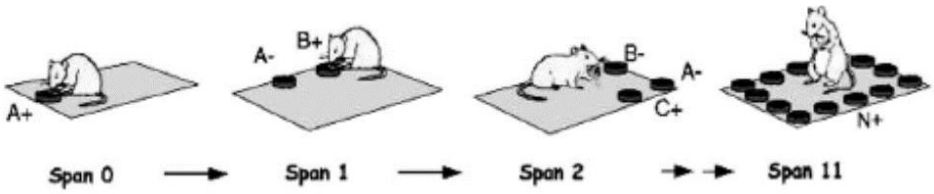


Fig.2.3.1. Odour span task, a rat is first presented with a cup of sand scented with a specific spice (**A+**), after a delay period it is presented the same scented cup of sand and a new cup of sand scented with another spice, food reward is available in the cup with the novel scent (**B**+), after a delay period, three cups of sand are presented, two with scented with the previous scent and another cup with a novel scent, food reward is presented in cup with the novel scent (**C+**). Additional cups are presented to rat, and its task is to remember which scents it has previously sampled. The rat's span is the number of odours it can correcty remember before making an error (Dudchenko 2004).

2.3.2. Novel object recognition task

Ennaceur and Delacour in 1988 (Ennaceur and Delacour 1988) devised a task in which spontaneous exploration of the objects was assessed: Novel Object recognition (NOR). In this task, a rat is presented with a pair of identical objects and left freely to explore the two objects. After a brief period of exploration, the rat is removed for a delay period and one of the object is replaced with a novel object. Then rat is brought back into the arena and the two objects are

presented. The rats' natural tendency is to explore more the novel object compared to the familiar one (**Figure 2.3.2.**).

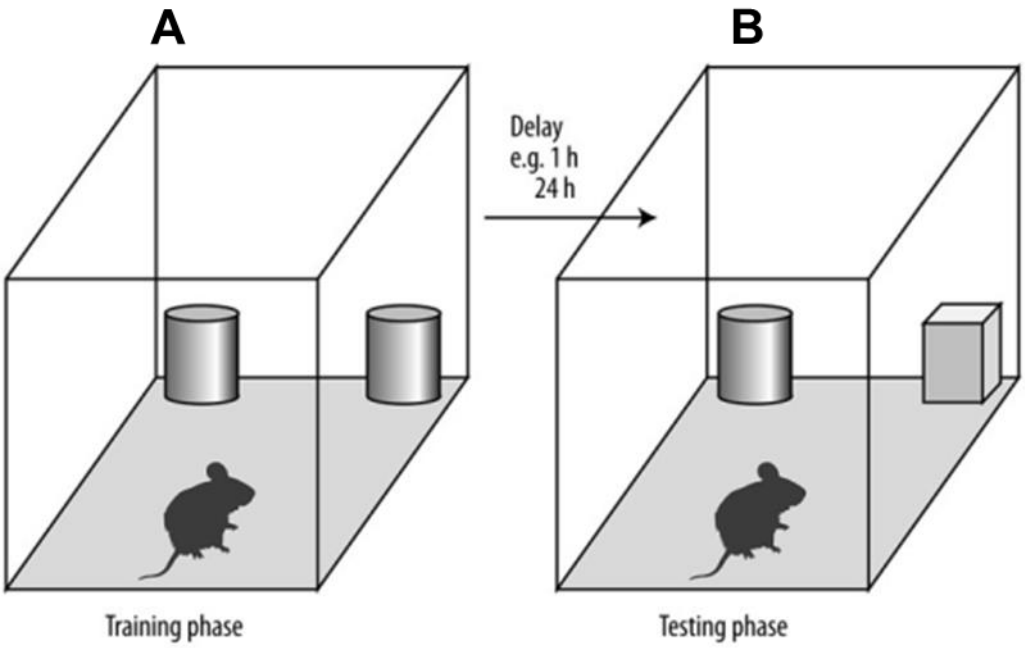


Fig.2.3.2. (**A**) Novel object recognition task. Rat is presented with two identical objects. (**B**) After a delay period one of the objects is replaced with a new one and the other with an identical copy. Rats usually tend to explore more time the new object compared to the familiar object.

In their task Ennaceur and Delacour used a delay period from 4 h to 24 h and observed that the preference for the new object is maintained for delays up to 4 hours and decrease after 24h. An important factor in this task is the exploration time given to rats to explore the two identical objects in first phase. If the exploration time is only 20 sec, the preference for the novel object is not significant after a 1 hour of delay.

2.3.3. 6 - different objects task and 6 - identical objects task

NOR test do not allow to study the object WMC in rodents. Sannino et al., 2012 (Sannino, Russo et al. 2012) proposed a new version of the NOR, in which the number of the object is increased to 3, 4 ,6 and 9 different objects. This allowed them to discover that the WMC in mice is six. The test that allowed them to define WMC in rodent as six is called 6 different object task (6-DOT). This task is used to asses object WM but in HML conditions. In the first phase, a mouse is presented with six different objects and it is left freely to explore the objects for 10 minutes or

for a 210 sec of total exploration. After that, the mouse is removed from the arena, and a delay of 1 minute is introduced. One of the objects is replaced with a new object, while the others are replaced with identical copies to prevent the presence of scent of the mouse. After a delay of 1 minute, the mouse is brought back to the arena and left freely to explore the objects for five minutes. As well as for the NOR, the mouse spent more time exploring the new object compared to the familiar objects. In the 6 identical object task (6-IOT), the mouse is presented with 6 identical objects and is left freely to explore the 6 object for 5 minutes or for a total exploration of 35 sec. After the mouse is removed from the arena and a delay of 1 minute is introduced. One object is replaced with a new object, while the other objects are replaced with copies in order to prevent the presence of mouse scents. After a delay of 1 minute, the mouse is reintroduced in the arena and left free to explore the objects. The natural tendency of the mouse is to explore more the new object compared to the familiar objects. The 6-IOT is considered a control task of the 6-DOT because in 6-IOT the memory load is low being all the objects identical. Whether a mouse is impaired in performing the 6-IOT, is very difficult that it is able to perform the 6-DOT **(Figure 2.3.3.)**.

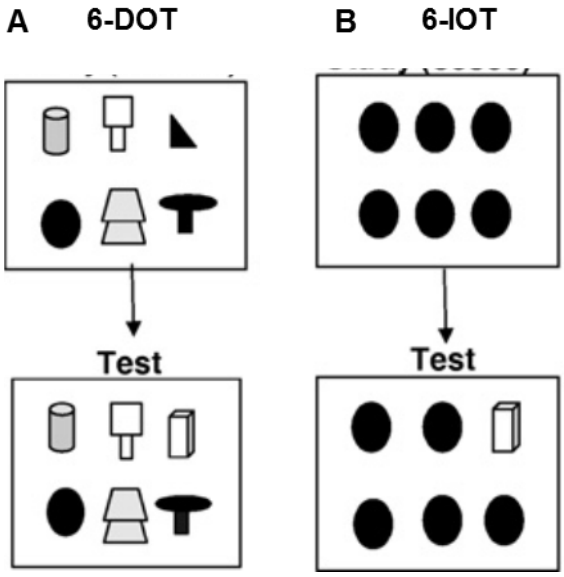


Fig.2.3.3. 6-DOT (**A**), mouse is presented with 6 different objects for 10 minutes or for a total exploration of 210 sec, after a delay period of 1 minute, one object is replaced with a new one and the other objects are replaced with copies.6-IOT (**B**) mouse is presented with 6 identical objects in the study phase for 5 minutes or a total exploration of 35 sec, after a delay period of 1 minute, one object is replaced with a new one and the other with copies. 6- DOT is an object working memory test in high memory load condition, while 6-IOT is an object working memory test in low memory load conditions (Sannino, Russo et al. 2012).

2.3.4. The radial arm maze

Olton and Samuelson in 1976 devised a task to assess WM in rodents, the radial arm maze. It is composed of eight arms radiating form a central platform. In their experiment, a rat is placed in the center of the apparatus, with a food reward at the end of each arms of the maze. Olton and Samuelson observed that rats are able to retrieve food from each arm, and quickly learn to visit all arms without entering in previously visited arms. First, rats are habituated to the apparatus placing them in the central platform and allowing them to freely explore the apparatus for 15 min per day. Reinforcement (food pellet) are scattered on the floor and rats can retrieve the food. On the last day of habituation the reinforcements are reduced to the half and the session ends when all eight arms are visited. After the habituation phase, rats are trained one session per day for eight consecutive days. The reinforcement is placed at the end of each arm in a well, hidden from the sights of the animals. Rats are free to explore the maze to retrieve food. Each session lasts until **1)** all eight arms are visited (a visit is considered when the animal enters in the arm with the whole body, except the tail); **2)** a fixed time (which is different form a protocol to another) elapses In order to prevent odour cues the maze is cleaned with wipes between different animals. The parameters analysed are **1)** The number of errors in each session (a re- entering in an arm previously visited is considered an error), and the total number of errors across the eight sessions; **2)** The number of correct choices in the first eight arms of each session **3)** The location of the first error in each session; **4)** The number of adjacent arms entries in each session; **5)** The time spent to visit (total time to complete the session divided by the total number of arm entries; **6)** The number of sessions to reach the criterion of one error or less. In this protocol rats usually enter > 7 arms before making an error (**Figure 2.3.4.)**.

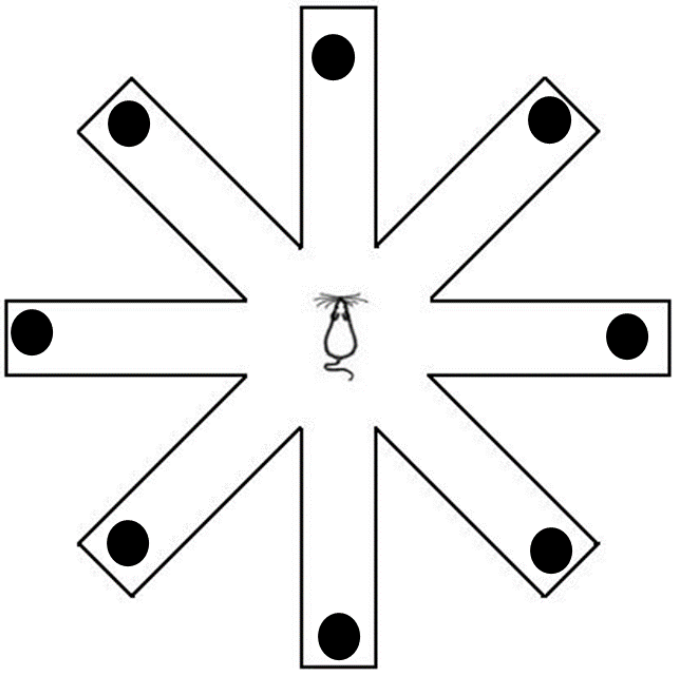


Fig.2.3.4. Eight arms radial maze. The mouse or rat is placed in the center of the apparatus and it is allowed to freely explore the apparatus to retrieve food reward (black circles) at the end of each arm of the maze.

In other experiments Olton and Samuelson tested rats in a version of the maze in which a delay was introduced after the rats had made the third arm choice. After a confinement period of 1 minute in the center of the apparatus, closing all the arms with guillotine doors, rats are allowed to freely explore the other 5 arms of the maze. Olton and Samuelson found that this delay had no effect on the accuracy of the test, indeed rats made an average of 7.7 correct responses in their first 8 choices. Increasing the delay at least at 2 minutes did not produce an impairment in the performance, indeed rats made an average of 7.6 correct responses in their first 8 choices. Based on this protocol Suzuki et al in 1980 introduced a delay of 2.5 min between the third arms choice and the subsequent choices. After this delay rats visited the remaining 5 arms they had not previously entered with an average of correct responses of 4.3 correct choices from the five remaining arms. They also found that rats based on extramaze cues to solve the task and the rearrangement of the spatial cues during the delay period resulted in chance performance following the delay. Bolhuis et al.1986 (Bolhuis, Bijlsma et al. 1986) studied how long could be the delay to impair rats' performance. They found that rats were impaired in solving the task with a 60 s delay between the fourth and subsequent arms choices and performed at chance level with 120 sec of delay. Jarrard et al in 1983 developed a version of the radial maze to test both WM and RM. In their version of the radial maze, only four of the eight arms were baited with food. The same arms were baited each day and across sessions. The rats learned to not enter in the four non - baited arms. This is the reference component of the task, and an entry into a never-

baited arm is considered a reference error, while a re - entry in a baited arms is considered a WM error. Jarrard et al in 1983 (Jarrard 1983) also tested rats in a match – to - sample version of the radial maze. In this task, the rat is allowed to explore the maze to find the reward. When it returns in the center of the apparatus is confined there for a delay period. After the delay, the doors of each arm are opened and rat is required to return in the arm where it obtained the reward. On different days, different arms are reinforced as are used as to be - remembered stimulus. But there is a problem: animals can solve the task in ways that not rely on spatial WM. One of these is the use of the chaining, serial or sequential strategy (entering each arm successively in a systematic order). To prevent the development of the sequential strategy one way is to interpose a delay between arm choices. This requires that after a subject enters an arm, all remaining doors are closed to prevent an immediate entry into another arm. Once the animal renters the center, the door of the arm visited is also blocked and the animal is confined in the center for a delay period. After the confinement is over, all the doors are raised and the animal can continue to freely explore the maze. This procedure is repeated each time an animal exits from an arm. The confinement procedure allows the animal to hold in working memory the last arm visited. Dubreuil et al. in 2003 (Dubreuil, Tixier et al. 2003) in their experiment, used confinement procedure to prevent the development of the sequential strategy. They use three different delay periods: 0, 5 and 10 s and demonstrated that even a confinement delay of 0 sec is sufficient to prevent the development of the sequential egocentric strategy, (a description of the difference between the allocentric and egocentric strategy will be provided in the next paragraph). Another strategy used to solve the task is the alternating strategy (Dubreuil, Tixier et al. 2003), in which a mouse enters in successively into arms separated by two arms (alternating strategy). The number of arms in the radial maze can be varied from four-maze which is called plus maze used by Olton and Feustle in 1981 (Olton and Feustle 1981) Other versions can include maze with 12,17 and 24 arms, which are used especially to study both WM and RM.

2.4. Types of navigation: allocentric and egocentric

Navigation is the ability of organisms to find their way in the environment without getting lost, and requires information for locations and routes. Two types of navigation can be distinguished: allocentric and egocentric. Allocentric way finding is the ability to navigate using distal cues and landmarks present in the environment, located outside the organism. Egocentric way finding is defined as the ability to navigate using internal cues (feedback from limb movement for rate of movement, direction, turns and sequence of turns). Egocentric navigation can be made in

darkness, indicating that the visual cues are not essential; even if egocentric navigational accuracy is reduced. By contrast, allocentric navigation cannot happen in the absence of distal cues (**Figure 2.4.**).

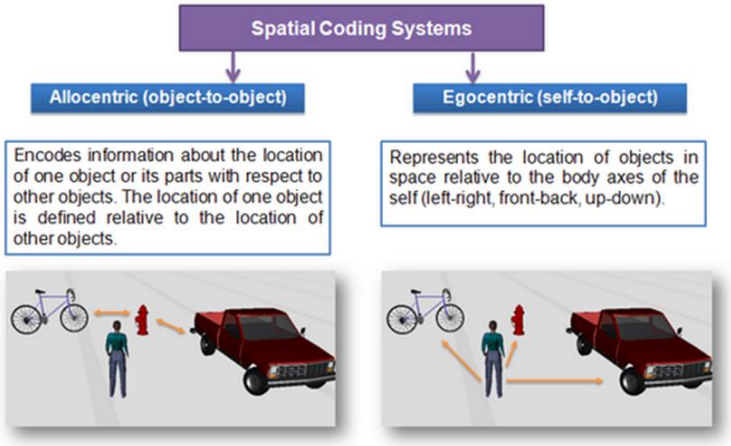


Fig.2.4. Schematic representation of allocentric and egocentric navigation.

In general, egocentric navigation is the ability to navigate by internal self - movement cues and can be divided in route - based navigation and path integration. Route- based navigation relies on internal cues of rate movements, turns and signposts, while path navigation involves the vector addition. Simply in a route - based navigation an organism follows a path with the order of turns remembered as set of specific rules, such as straight, left, right, left, right, left. These memorized operation can became habits. Path - integration is the ability of an organism to leave its home - base and move to different locations and then return by a different, more direct path. What are the brain region involved in navigation? In the allocentric navigation, the regions more involved are the hippocampus and the entorhinal cortex. With electrophysiological studies, place cells were identified in the hippocampus (Nadel 1978, O'Keefe and Conway 1978). These cells respond to different environments and features within them. They form a map of the environment and they remap the environment as the organism moves within a space or moves in another space. Also in the medial entorhinal cortex place cells have been identified and the entorhinal cortex communicate with hippocampus (Hafting, Fyhn et al. 2005). In the entorhinal cortex are also present grid cells. Each grid cell is characterised by spacing (distance between fields), orientation (tilt relative to an external reference axis) and phase (xy displacement relative to an external reference point). The smaller response field are located in the dorsal entorhinal cortex, while the larger response field are located in the most ventral region of the enthorinal cortex. In the entorhinal cortex, in presubiculum and parasubiculum head direction cells are

present (Buzsaki and Moser 2013), that have a role in orientate an organism to distal cues and contribute to the direction of the movement. In the entorhinal cortex, border cells are also present (Solstad, Boccara et al. 2008), these cells have response fields that react to the boundary and the edges within the environment. Place, grid, head direction cell, and borders cells in the entorhinal cortex and place cells of the hippocampus form a network that map places in the space outside the organism. Brain regions involved in the egocentric navigation are less known. An important fact is that allocentric and egocentric navigation can overlap, because head direction cells are very important for egocentric navigation. They are present in other brain region as thalamus, mammillary nucleus, retrosplenial cortex and dorsal striatum. Sometimes allocentric and egocentric navigation could be dissociated indeed for example hippocampal lesion causes spatial but not nonspatial impairment in the Morris water maze or dorsal striatum lesions cause nonspatial impairment in the Morris water maze (Packard and McGaugh 1992, McDonald and White 1994, Devan, McDonald et al. 1999). Experiments in humans with virtual reality demonstrated that egocentric path integration recruits neural activity in the hippocampus and parietal cortex (Sherrill, Erdem et al. 2013).

2.5. Morris Water Maze

Richard Morris developed Morris Water maze in 1984. It is a test for spatial learning in which rodents have to use distal cues to create a map to find an hidden platform in an open swimming arena. In the basic and classical procedure of the Morris water maze, rodents have to learn, using distal cues, the path to find an hidden platform, starting from four different location: south, north, west and east. The training lasts 5 days with 4 trials per day. To assess RM, a probe test is done the subsequent day of the last training day. In the probe phase the platform is not present (**Figure 2.5**.).

Fig.2.5. Schematic representation of the Morris Water Maze. Rats or mice have to find an hidden platform, using spatial cues present in the environment.

A variant of the test is the spatial reversal in which the platform is relocated in another quadrant (usually the opposite quadrant), and rodents have to made other 5 days of training with 4 trials per day. Reversal learning is useful because it can test the ability of rodents to acquire a new path to reach the platform. In the spatial double-reversal with a smaller platform the platform is moved back to the original goal (double – reversal) or to a different quadrant (shift), but with an additional change: the use of a smaller platform. For example if the starting platform was 15 x 15 cm, the reduced platform is 10 x 10 cm. This reduction in platform size allows investigating the spatial accuracy requirements of the rodents. Another procedure is to perform a set of reversal and shift phases serially. This allows investigating the animal's flexibility to learn across multiple phases of learning. Morris water maze is also used to test spatial WM, in this case, a matching- to sample method is used. The platform is relocated each day, with two trials per day. In the first trial the animal, have to learn the new location of the platform by trial and errors. The second trial is the test or matching trial in which savings in recall the between trial 1 and trial 2 are measured. If the animal recall the sample trial, it will swim with a more direct path to the platform. A control condition which is very used in the Morris water maze, is the cued learning. The platform is the same as used in the hidden version, but it is elevated above the water surface and usually a flag is mounted on it. The location of the goal and start are both moved to new position during each trial. In this version the platform is visible to rodents and this test is used to assess same basic abilities as (intact eyesight, motor ability), basic strategy (learn to swim away

from the wall, learn to climb on the platform). If a rodent is impaired in the cued version, it will be difficult for it to learn spatial version.

3. Aim of the study

Working memory (WM) refers to a brain system that provides temporary storage and manipulation of information necessary for complex cognitive tasks as language comprehension, learning and reasoning (Baddeley and Hitch, 1974). An important concept in WM is its capacity or WM span, that is the amount of information that one subject can retain for a short period and it is estimated to be 7± 2 in human (Miller, 1956). WM alterations are associated with a wide range of deficits such as attention deficits, or problems in the manipulation of the information which could result in problems in daily life as carry on a conversation. Traditionally WM was associated with dopaminergic fronto - striatal network, whose deregulation causes severe alterations in WM (Klostermann, Braskie et al. 2012). In the last decades, a role of the hippocampus in WM has been investigated, but the data are controversial. Studies on rhesus monkey with a lesion in the hippocampus have shown a performance similar to control when the number of item to- be-remembered was high (Murray and Mishkin 1998), while other studies showed a deficit in the performance in rhesus monkey with bilateral lesions of the hippocampus only in high memory load conditions (Beason-Held, Rosene et al. 1999). Similar findings were found in a recent study in rodents (Sannino, Russo et al. 2012) in which it was reported that dorsal hippocampus lesioned mice were impaired in the discrimination of a novel object in high memory load (HML) conditions, using a task in which animals had to discriminate a new object among 5 familiar objects (the 6 different objects task/6-DOT). All toghether these findings suggest that the hippocampus regulates working memory capacity (WMC).

The hippocampus is a heterogeneous structure, with its dorsal region defined as a "cold hippocampus" more involved in cognitive processes as learning, memory, navigation and exploration, while its ventral part defined as a "hot hippocampus", which is more involved in the processing of emotional information (Fanselow and Dong 2010). There are no studies in literature which investigate the role of dorsal and ventral hippocampus in WMC. The aim of this study is, therefore, to investigate the role of dorsal and ventral hippocampus in spatial WMC and object WMC. We selectively lesioned dorsal and ventral hippocampus with high concentration of N-methyl-D-aspartate (NMDA); after lesion mice were tested in a modified version of the eight arms radial maze in which the memory load was changed by changing the number of open /baited arms between trials and among days with 3 open/ baited arms (low memory load

condition), 6 open/baited arms (intermediate memory load condition) and 8 open /baited arms (high memory load condition). Mice usually solve the task using an egocentric sequential strategy which reduces the WM load. Therefore, in order to assess WMC we introduced a confinement procedure that has been previously showed to prevent the use of egocentric strategies (Olton 1977, Dubreuil, Tixier et al. 2003)

After five days of confinement procedure, we switched to the no - confinement procedure, to observe whether there were changes in the use of strategy used to solve the task. Finally, to assess the role of these distinct two regions in object WMC in HML conditions, the same mice were tested in 6-DOT/IOT. There is no evidence in literature whether there is a functional difference between ventral and dorsal hippocampus in object WMC. The results of this study are highly relevant for humans, as reduced WMC is a core cognitive symptom of schizophrenia and ageing.

4. Materials and Methods

4.1. Subjects

CD1 outbred male mice (Charles River); 5-7 weeks on arrival are used for all experiments. They are housed five per cage with food and water *ad libitum.* Mice are kept on 12 hours light/dark phase and tested during the light phase (9:30 a.m-6:00p.m). Before testing animals are acclimatized to the behavioral room for at least 30 minutes. At the beginning of the experiment mice are 15-20 weeks old. All procedures related to the animal care and treatment are conformed to the guidelines and policies of the European Communities Council and approved by the Italian Ministry of Health.

4.1.1. Surgery

All surgical procedures are performed under general anaesthesia using avertine injected intraperitoneally (i.p.). Mice are placed in a stereotaxic frame (Kopf Instruments, USA). N-methyl-D-aspartate (NMDA, Sigma Aldrich, Italy), (20 mg/kg) is injected bilaterally in the dorsal and ventral hippocampus with a volume of 0.3 µL/side. The stereotaxic coordinates for the bilateral lesion of the dorsal hippocampus are anteroposterior (AP) =-1.9 mm, lateral (L) =± 1.2 mm, dorsoventral (DV) = -1.6 mm. For the bilateral lesion of the ventral hippocampus are

AP=-3.3 mm; L=-3.0 and DV=- 3.7 mm from the bregma. The stereotaxic coordinates are taken according to the atlas of Franklin and Paxinos (1998). The sham group receives a bilateral injection of PBS 1X at the same coordinates. After surgery, mice are allowed to recover for ten days before behavioral tests. At the end of all behavioral procedures, mice are deeply anesthetized and transcardially perfused with phosphate buffered saline (PBS 1X, pH 7.4), followed by 4% paraformaldehyde in PBS. Brains are removed and post- fixed in the same fixative for a week and then cut on a vibratome. Only animals with correct placements, verified under a light microscope by analysing consecutive coronal brain sections (50µm) stained with Nissl staining, are included in statistical analysis.

4.2. Behavioral procedures

4.2.1. Elevated plus maze apparatus and procedure

The elevated plus maze is widely used in literature to test anxiety and to validate new anxiolytic drugs because it has a predictive and construct validity. It is based on the natural aversion of rodents to heights. The apparatus consists of four arms (37 x 9cm), two open arms and two enclosed arms and lit by a 100 W light. The maze is elevated from the floor 50 cm. The mouse is placed in the center of the maze, at the junction of the four arms of the maze, and it is free to explore the maze for 5 minutes. The test is videotracked by a camera mounted on the ceiling and connected to a videotracking system (AnyMaze, Stoelting, USA). The measures taken are , the entries in the open arms, the percentage of time in the open arms, the distance in open arms and the total distance travelled. More time spent in the open arms is an index of less anxiety.

4.2.2. The 6-DOT/IOT tasks apparatus and procedure

The 6-DOT is a modified version of the NOR. It is used to test object WM in HML conditions (Sannino, Russo et al. 2012). In the 6-DOT mice are isolated for 15 minutes in a waiting cage before testing and then subjected to a habituation period of 10 minutes in an empty arena (35 x 47 x 60 cm), T1 phase. Habituation period allows assessing motor impairment. After 1 minute of inter trial interval (ITI) spent in their waiting cage, mice are subjected to the testing phase, T2, during which they explore six different objects for ten minutes or for a total exploration of 210 sec. Exploration is considered when mouse approaches to the object at a distance of 2 cm. In the

last phase, T3, after 1 minute of ITI in their waiting cage, the objects are replaced with identical copy of the familiar objects and a new object for 5 minutes. The six identical object recognition task (6-IOT), is considered a control test, in this case WM load is low, because mice have to discriminate among identical objects. T1 phase is identical of that of 6 DOT, in the T2 phase mice are allowed to explore objects for a total exploration of 35 sec or for a total of 5 minutes In the T3 phase, all the objects are replaced with identical copies of the familiar objects and a new one for 5 minutes.

4.2.3. Morris Water Maze apparatus and procedure

Morris Water Maze apparatus is a circular pool (110 cm diameter x 36 cm depth), filled with water at a temperature of 22°C. The pool is filled with water with black no toxic colour, (Helios, Milan), until 10 cm from the edge. The black colour is used to make invisible the platform and to increase the contrast between the mice and the apparatus. It is important to create a contrast between mice and water in order to allow to videotracking system to follow mice in their path to reach the platform (13 cm diameter), which represents the escape from water. The apparatus is ideally divided in four quadrants; north, south, west and east. The platform is located in the south quadrant during the shaping phase and in the north quadrant during the training phase. Around the platform area, the videotracking system creates a concentric circle (annulus) of a diameter of 25 cm. The test is video tracked by a camera mounted on the ceiling and linked to a videotracking system (AnyMaze, Stoelting, USA). The apparatus is illuminated by a 100 W light, and a by a neon light, and enclosed by a grey curtain at south and west. The apparatus is surrounded by different spatial cues, which are maintained in a fixed position during the test. The behavioral procedure is a modification of the protocol described in Ferretti et al., 2007. It consists of three phases: shaping phase, training phase and probe test. In the shaping phase, the platform is visible (1 cm above the water surface), and is located in the south quadrant. It consists of three consecutive trials in which mice are released from three different quadrants, choosed in a pseudo-random way. The platform is visible to habituate mice to the new environment, reducing the emotional component (stress, anxiety), which can influence the performance of the test. The localization of the platform is different from that used in the training phase in order to avoid pre - learning in this phase. Mice are isolated in their waiting cage for 30 m before any behavioral procedure. Shaping phase starts putting the mouse on the platform for 60 s, in order to allow it to observe the behavioral room and to habituate to the platform. At the start of every trial mouse is released in the pool with its face toward the pool

wall. AnyMaze records the path of the mouse to reach the platform. Every trial has a cut-off of 60 s. If the mouse reaches the platform it is allowed to stay on it for 15 s, if it does not reach the platform, it is gently accompanied by the experimenter on the platform and allowed to remain on it for 15 s. The quadrants in which the mouse is released are chosen in a pseudo-random way in order to avoid that starting from the same quadrant, mouse can learn a specific path using an egocentric strategy, without using the spatial cues present in the environment. In the second day, the training phase starts. The platform is located in the north quadrant, and it is submerged 0.5 cm below the water surface. It consists of six sessions of three consecutive trials per sessions, with an ITI of 30-45 m, in which mice are returned in their waiting cage. If a mouse does not reach the platform, the experimenter gently accompanies it and it is allowed to remain on it for 15 s. After the three consecutive trials end, mouse returns in its waiting cage and another mouse is tested. The six sessions are made using the same procedure, the starting quadrants change from a session to another, and they are chosen in a pseudo-random way. On the following day, 24 hours later, probe test takes place, the platform is removed and mouse is released in the center of the pool and videotracked for 60 s. The measures analysed in the training phase are the time spent to reach the platform (latency) and the distance travelled to reach the platform. In the probe test the percentage of time in the target quadrant (the quadrant in which the platform was located) is analysed and the entries in the annuli.

4.2.4. Eight arms radial maze apparatus and procedure

The radial arm maze apparatus is constructed with clear Plexiglas material with painted grey flooring. It consists of eight equally spaced arms (38 cm in length, 8 cm in width, 9 cm in height), all radiating from a small octagonal central platform (with a diameter of 19 cm). Plexiglas doors of 9 cm in height are placed if necessary between every arm and the central area. The maze is elevated 84 cm from the floor on a platform It is enclosed by curtains hung from the ceiling in one corner of the behavioral room and is illuminated by overhead white lighting. Four visual cues have been positioned around the maze. The experimenter is in the same fixed position for all the duration of the test. The confinement box is hand made, created with three clear Plexiglas squares, glued together. It is placed at the end of the arm as soon as the mouse enters in it, so when it is leaving the arm, goes in the box and the Plexiglas door of the arm closes the box. The mouse is confined in the box for 5 seconds. Then, the box is raised to allow the mouse to continue to explore the maze. The day before the start of the test mice are food - restricted so they reach and maintain 80-85% of their free feeding weight for all the duration of

test. The test consists of three day of habituation to the apparatus for ten minutes. During the habituation phase, all arms are open and twenty chocolate cereal grains are placed in the apparatus (two for every arm, one at the entrance and one at the end of every arm and four in the central zone). The number of cereal grains eaten is recorded. On the second day of habituation, the confinement box is introduced to habituate them to it. In the habituation period mice do not enter automatically in the box, so when they are almost at the end of the arm, the Plexiglas door of the arm is gently placed between the mouse and the box, so the mouse is confined and enters the box. In the training phase, mice do not longer consider the box as "something adverse" and go straight to it. After the habituation period, a pretraining phase (PT) takes place. It consists of nine trials per day for two days. During the PT phase only two of the eight arms are open and baited. The open/ baited arms change within the trials and between the two days. Mouse is placed in the center of the apparatus and the stopwatch is running. Every time the mouse enters an arm, the experimenter places the confinement box at the end of the arm and when the mouse is in the box, closes the door of the arm. The mouse is confined for 5 seconds. During the trial, the experimenter records the path of the mouse and the time when the mouse enters the last baited arm. The trial is considered completed when the animal has visited the two different arms or 6 minutes have passed. After the PT phase, a training phase takes place. It consists of nine trials per day for five consecutive days, in which the number of open/ baited arms change within trials and among days (3, 6 and 8 open/baited arms). In these first five days, the confinement box is used (confinement procedure). A trial ends when a mouse enters all the open/baited arms or when 6 minutes have passed. After the five days of confinement procedure, four days with no - confinement take place. They consist of four days of training, without confinement box, with nine trial per day. A trial ends when a mouse enters all open/baited arms or 5 minutes have passed. It is important to clean the apparatus after every trial with 25% ethanol solution, in order to eliminate the scents of the mouse and of the experimenter. All the experimental group is tested for the first trial, before performing to the next one. The measure analysed in the PT phase is the mean number of errors. In the training phase in both confinement and no - confinement procedure, the measures analysed are: the mean number of errors in the confinement and no - confinement procedure, the mean of the score of the sequential strategy at 3, 6 and 8 open/baited arms in the confinement and no - confinement procedure, the mean of the score of the alternating strategy at 3, 6 and 8 open/baited arms in the confinement and no- confinement procedure.

4.3. Statistical analysis

Elevated Plus Maze: control mice= 11; dorsal hippocampus lesioned mice= 8; ventral hippocampus lesioned mice= 16

We analyse the time spent in open arms, the total distance travelled, the percentage of the time spent in open arms and entries in the open arms using a one-way ANOVA using as the between factor the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice).

6-DOT/IOT: control mice = 8; dorsal hippocampus lesioned mice= 6; ventral hippocampus lesioned mice = 12

The analysis of distance in T1 and the exploration in T2 are performed with a one-way ANOVA, using as a variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice). The analysis of exploration in T3 is made using a two- way ANOVA for repeated measures using as a variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice), and as repeated measures the number of objects (six levels: six objects). Novel object discrimination is defined as: the new object explored significatively more than the all other familiar objects based on the results of Duncan *post - hoc* analysis.

Morris water maze: control mice = 12; dorsal hippocampus lesioned mice = 10; ventral hippocampus lesioned mice = 15.

The mean of latency and distance is made with an average of the values of three trials, in order to obtain a single value of latency and distance. The analysis is performed with a two- way ANOVA for repeated measures using as a variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice), and the variable sessions as repeated measure (six levels: six sessions). A significative reduction in the mean latency or in the mean distance represents an index of learning. In the probe test, the analysis of the percentage of the time spent in the quadrant is performed with a two-way ANOVA for repeated measures using as variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice) and as repeated measures the percentage of time spent in each quadrant (four levels: north, south, west and east). The entries in the annulus are analysed with a two-way

ANOVA for repeated measures using as variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice) and as repeated measures the factor entries in the annuli (four levels: north, south, west and east).

Significance is set at $p<0.05$. All the effects are decomposed with Duncan *post – hoc* analysis.

Eight-arm radial maze: Two control animals are tested in the confinement procedure and not in the no - confinement procedure with a total of 10 control mice in the confinement procedure and 8 control mice in the no - confinement procedure; dorsal hippocampus lesioned mice = 8; ventral hippocampus lesioned mice = 12.

In the PT phase, the mean number of errors is analysed with a one-way ANOVA with treatment as between variable (three levels levels: control, dorsal and ventral hippocampus lesioned mice).

Training phase: The analysis of the mean number of errors at 3, 6 and 8 open/ baited arms is performed before making an average of the number of errors for the five days of confinement procedure and a mean for the four days of no - confinement procedure and then analysed with a three-way ANOVA for repeated measures, using as variable between the factor treatment (3 levels: control, dorsal and ventral hippocampus lesioned mice), and as repeated measures the number of open/baited arms (3 levels: 3, 6 and 8 open/baited arms) and the procedure used (two levels: confinement/no - confinement).

The analysis of the mean number of errors at 3, 6 and 8 open/baited arms for the dorsal hippocampus lesioned mice and the ventral hippocampus lesioned mice separately is made with a three- way ANOVA for repeated measures using as variable between the treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice) and as repeated measures the factor number of open/ baited arms (three levels: 3, 6 and 8 open/baited arms) and the factor procedure (two levels: confinement/no - confinement). Significance for the treatment is performed using a two- way ANOVA for repeated measures using as variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice), and as repeated measure the number of open/baited arms (three levels: 3, 6 and 8 open/baited arms). Duncan *post – hoc* analysis is applied on the two-way ANOVA.

Calculation of the score of sequential strategy: The score of sequential strategy is calculated by counting +2 when a mouse enters an adjacent arm. This is called sequential strategy with errors because it does not take in account the re - entry in a previously visited arm. For example

in the sequence of visits 1-2-3-4-5-6-7, where each number represents a visit in an arm, the total score is 7. But we take in account even when a mouse make an error (a re-entry in a previously visited arm), in this case we a score of -1 is given and it is subtracted to the total score of the sequential strategy. This is called sequential strategy without errors: for example for the sequence 1-2-3-4-5-6-5-, the total score of the sequential strategy is six with one error (the re-entry in the arm 5). The score of alternating strategy is calculated by counting +1 when a mouse enters in alternating arms, for example 1-3-5-7 , has a score of 4. The score are calculated automatically by a software, RAM Tigem, developed by the Tigem Bioinformatics core. The sequential and alternating strategy analysed in this work are the sequential and alternating strategy with errors.

The analysis of the mean of the score of the sequential and alternating strategy is performed before making an average of the score of sequential or alternating strategy for the five days of confinement procedure and the four days of no - confinement procedure and then is analysed with a three- way ANOVA for repeated measures using as variable between the factor treatment (three levels: control, dorsal and ventral hippocampus lesioned mice) and as repeated measures the variable number of open/baited arms (three levels; 3, 6 and 8 open/ baited arms) and the procedure (two levels: confinement/ no confinement). The analysis of the mean of the sequential or the alternating strategy for the dorsal and ventral hippocampus lesioned mice separately is made with a three-way ANOVA using as variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice) and as repeated measures the factor number of open/baited arms (three levels: 3, 6 and 8 open/baited arms) and the procedure (two levels: confinement/ no confinement). Significance for the treatment is analysed with a two-way ANOVA for repeated measures using as variable between the factor treatment (two levels: control and dorsal hippocampus lesioned mice or control and ventral hippocampus lesioned mice), and as repeated measures the number of open/baited arms (three levels: 3, 6 and 8 open/ baited arms). Duncan *post – hoc* analysis is applied on the two-way ANOVA.

The analysis of the sequential strategy compared to the alternating strategy is made using a four way ANOVA for repeated measures using as variable between the factor treatment (two levels: dorsal and ventral hippocampus lesioned mice) and as repeated measures the factor strategy (two levels: sequential /alternating strategy), the number of open/ baited arms (three levels: 3, 6 and 8 open/ baited arms) and the factor procedure (two levels: confinement/ no confinement).

Significance is set at $p<0.05$. All the effects are decomposed with Duncan *post – hoc* analysis.

The tests are performed in this order: elevated plus maze; 6- DOT, 6-IOT, Morris water maze, radial arm maze (**Figure 4**).

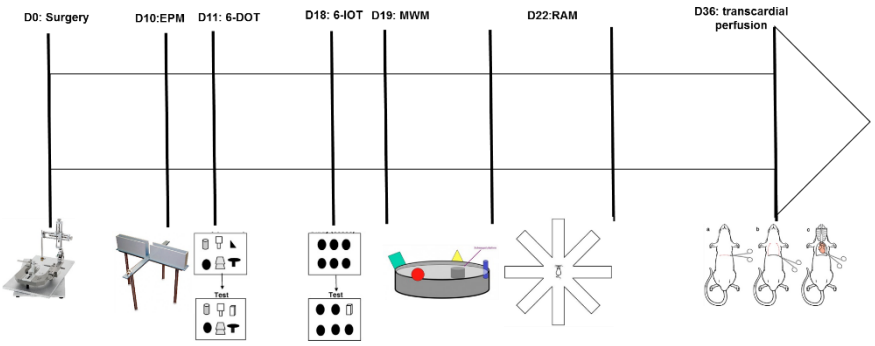


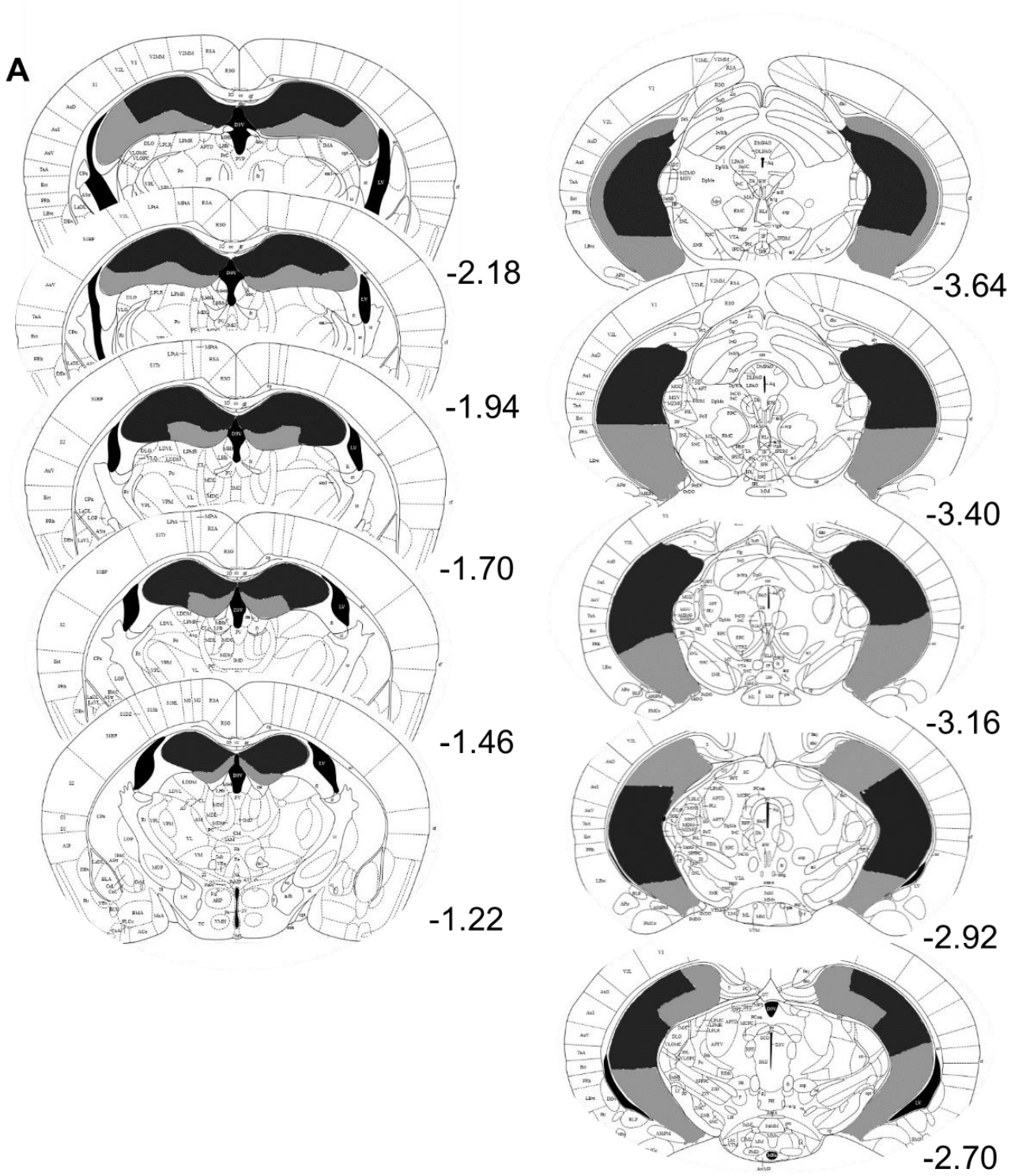
Fig. 4. Schematic representation of the experimental plan. Abbreviations: D: days; EPM: elevated plus maze; MWM: Morris water maze; RAM: radial arm maze.

5. Results

5.1. Histological verification of the lesion

Nissl staining on consecutive coronal section of the brain of lesioned animals, as compared to sham control animals, showed a wide cellular loss, with necrosis areas and alteration of the tissue. Anteroposterior analysis of the dorsal lesion, such reported in the Franklin and Paxinos, 1998 and relative to the bregma distance, showed that animals had a wide bilateral lesions which extended from the coordinates – 1.22 to -2.98, with the more extended lesion including wide neuronal loss and gliosis in CA1, CA2 and CA3 fields of the hippocampus and dentate gyrus, while the smallest lesion cover CA1, CA2 and CA3 with no dentate gyrus. The antero-posterior analysis of the ventral hippocampus lesion revealed that the lesion extended from the bregma -2.70 to -3.64 with the more extended lesion including CA1, CA2 and CA3 and dentate gyrus, while the smallest lesion cover a portion of the CA1, CA2 and CA3 and a small portion of the dentate gyrus, with no lesion in the entorhinal cortex (**Figure 5.1.**). Our approach of excitotoxic lesion with high concentration of NMDA is widely used in literature, for example Pothuizen in 2004 (Pothuizen, Zhang et al. 2004) performed excitotoxic lesion with NMDA in dorsal and ventral hippocampus of the rats, in which the dissociation of the two areas was made using our criterion; posterior hippocampus corresponding to the temporal/ventral hippocampus, while the

anterior portion corresponding to the septo/dorsal hippocampus. In their lesions Pothuizen found that dorsal hippocampus lesion is characterized by extensive cell loss and gliosis of all the three CA subfields of the hippocampus, CA1, CA2 and CA3 and also in the dentate gyrus. Our dorsal lesion is similar extending in the three CA subfields and in the more extended lesion in the dentate gyrus. Ventral lesion in Pothuizen are characterized by cell loss in all the ventral hippocampus and described two cases in which ventral lesions extended also in the temporal part of the dorsal hippocampus. Sometimes the lesion spared the CA1 subfield, and the lesion never extended in the enthorinal cortex as in our case. This approach and the dissociation of dorsal and ventral hippocampus based on antero-posterior bregma coordinates is used in Trivedi in 2006 (Trivedi and Coover 2006) in which excitotoxic lesion with NMDA is performed in rats. In their work they found that dorsal hippocampal lesion included all the three CA subfield and the dentate gyrus, and in some cases in dorsal subiculum. Lesion in the ventral hippocampus extended in the three CA subfields, dentate gyrus and almost in all cases in the ventral subiculum. Also in Bannerman in 1999 (Bannerman, Yee et al. 1999), it is reported that NMDA lesion in dorsal and ventral hippocampus in rat's brain, involved the three CA subfield, but in this case neither the dorsal nor the ventral subiculum. The anteroposterior distinction between the dorso and the ventral hippocampus has also been recently posed on a genetic basis by a study in our laboratory showing that developmental loss of function of the gene encoding for the gene COUP-TFI leads to a dramatic reduction of the volume dorsal/anterior hippocampus, while it spears the most posterior/ventral part (Flore, Di Ruberto et al. 2016).


A
-2.18
-1.94
-1.70
-1.46
-1.22
-3.64
-3.40
-3.16
-2.92
-2.70

B

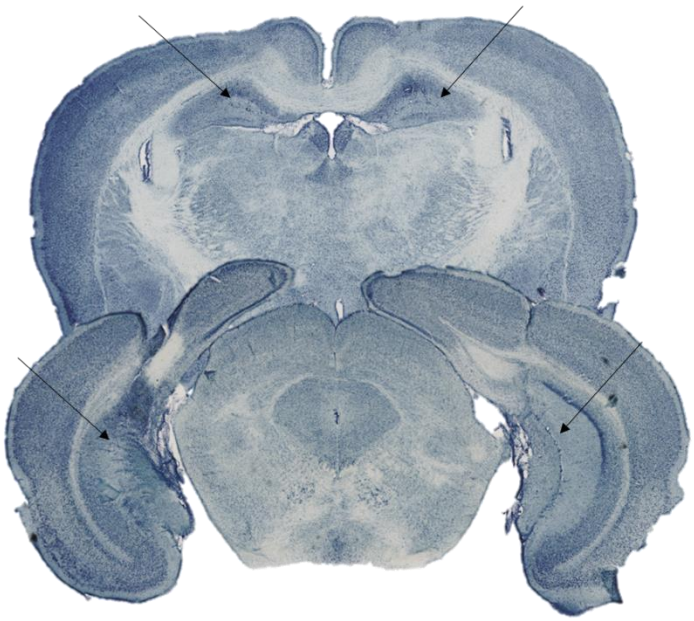


Fig.5.1. (**A**) **Grafic reconstruction of the dorsal and ventral lesion.** Panel of coronal sections taken from Franklin and Paxinos mouse brain atlas (1998). Numbers indicate distance in millimeters from bregma. The plates show the smallest representative (black) and the largest representative (gray) lesion in dorsal (left) and ventral (right) hp lesioned mice. (**B**) **Histological sections of a dorsal and a ventral hp lesion.** Photomicrographs of a representative dorsal (upper) and ventral (lower) hp lesion Nissl-stained. Black arrows indicate the lesions.

5.2. Effect of dorsal hippocampus lesion on anxiety

Performance in the elevated plus maze was tested to assess the level of anxiety in lesioned animals. One-way ANOVA showed that dorsal hippocampus lesion did not affect the percentage of time (**Figure 5.2.A**), [treatment ($F_{1,17}$=0.230;p=0.6373)], the distance travelled [treatment ($F_{1,17}$=1.850;p=0.1916)] (**Figure 5.2.B**) and the number of entries [treatment ($F_{1,\ 17}$=0.064; p=0.8038)] (**Figure 5.2.C**) in the open arms, as well as the total total distance travelled [treatment ($F_{1,\ 17}$=3.392; p=0.0830)] (**Figure 5.2.D**). All together, these data suggest that dorsal hippocampus is not involved in modulating anxiety in this task.

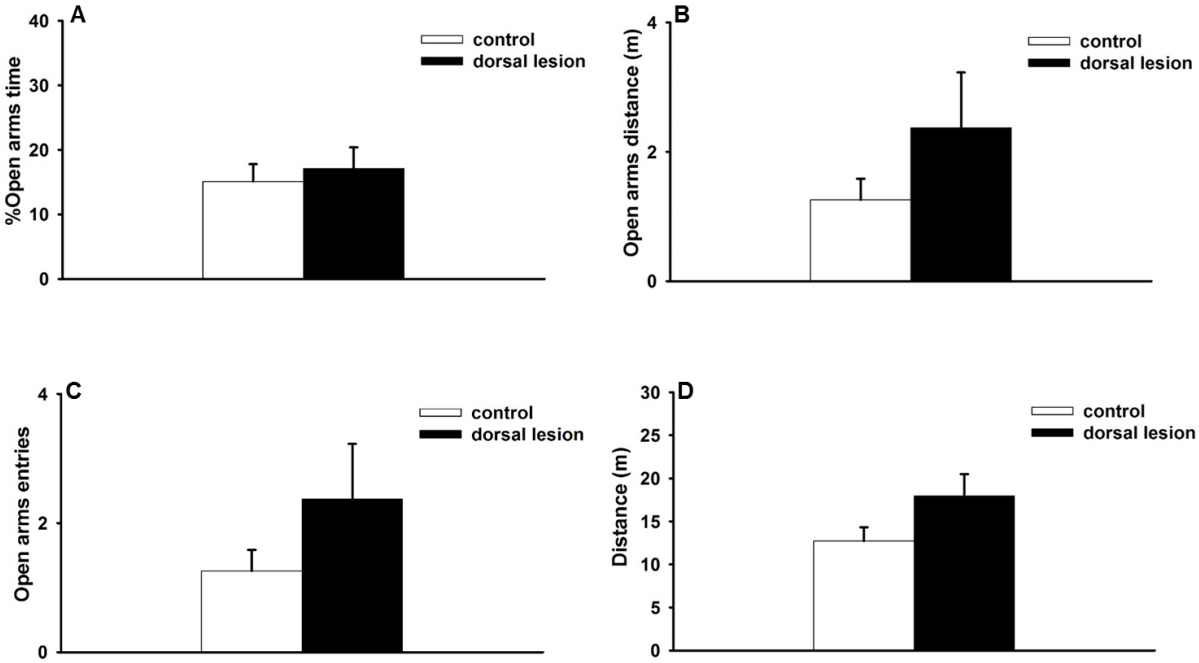


Fig.5.2. Elevated plus maze. (**A**) Percentage of open arms time for control and dorsal hp lesioned mice. (**B**) Open arms distance. (**C**) Open arms entries. (**D**) Total distance travelled. Data are expressed as mean ± SEM.

5.2.1. Effect of ventral hippocampus lesion on anxiety

Differently from what reported for the dorsal hippocampus, ventral hippocampus lesion increased the of the percentage time [treatment ($F_{1, 25}$=8.806; p=0.0065)] (**Figure 5.2.1.A**), the distance [treatment ($F_{1, 25}$=3.897; p=0.0595)] (**Figure 5.2.1.B**) and the number of entries [treatment ($F_{1,25}$=3.279;p=0.0822)] (**Figure 5.2.1.C**) in open arms, as compared to control, although only the first measure was fully significant. The one- way ANOVA for the analysis of the total distance travelled showed that there were no significant differences in the total distance between the two experimental groups [treatment (F1,25=0.011;p=0.9179)] (**Figure 5.2.D**). This analysis suggests that ventral hippocampus is more involved than dorsal hippocampus in anxiety and emotional processes.

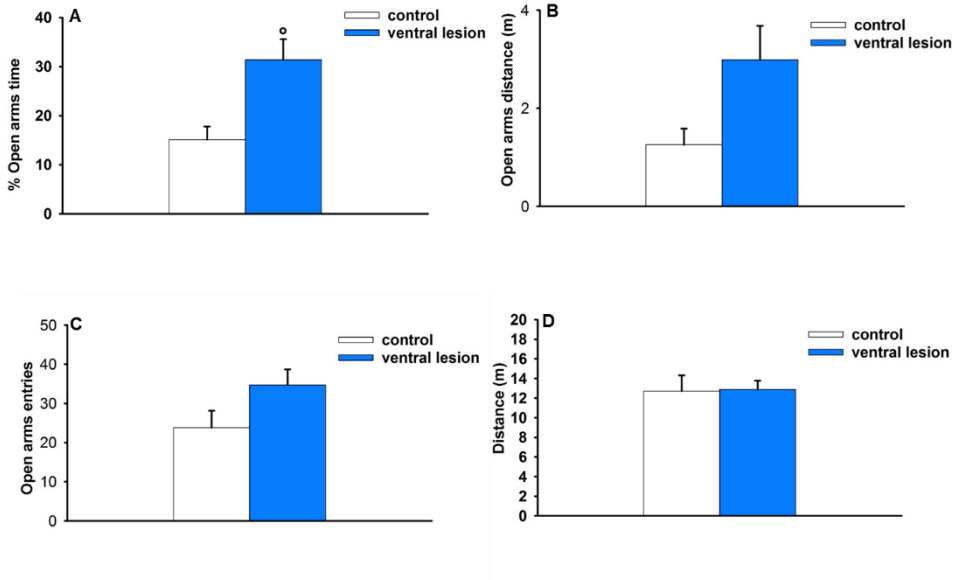


Fig.5.2.1. Elevated plus maze. (**A**) Percentage of open arms time for control and ventral hp lesioned mice. Data are expressed as mean ± SEM. °p <0.05 ventral hp lesioned mice vs control. Duncan *post - hoc* analysis. (**B**) Open arms distance. (**C**) Open arms entries. (**D**) Total distance travelled. Data are expressed as mean ± SEM.

5.3. Effect of dorsal hippocampus lesion on object WM in high memory load conditions

The 6-DOT consisted of three phases; first phase of 10 minutes (T1), in which mice were free to explore an empty arena, a second phase of 10 minutes or 210 sec of total exploration (T2) in which mice had to explore six different objects, and a last phase of 5 minutes (T3) in which one of the objects was replaced with a new one and the other objects, called familiar objects, were

replaced with identical copies. In the T1 we measured the total distance travelled in the arena, we did not find significant differences between control and dorsal hippocampus lesioned mice (**Figure 5.3.A**). In addition, we did not observe significant differences between the two groups in the total exploration (**Figure 5.3.B**). In Sannino et al. 2012 was reported that dorsal hippocampus lesions impaired object WM in HML conditions. We first replicated this result showing that control mice explored the new object significantly more than the familiar ones, while dorsal hippocampus lesioned mice were not able to discriminate the new object. This analysis confirm that dorsal hippocampus lesion causes an impairment in object WM in HML conditions as demonstrated in Sannino et al., 2012 (Sannino, Russo et al. 2012) (**Figure 5.3.C)**.

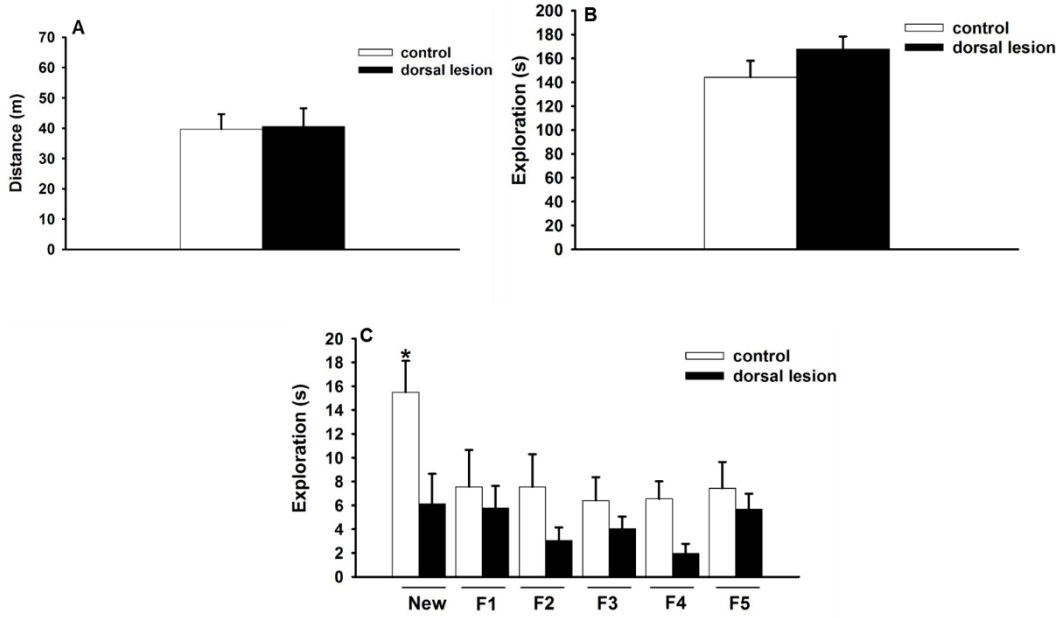


Fig.5.3. 6-DOT. (**A**) Total distance travelled in T1 phase in control and dorsal hp lesioned mice. (**B**) Exploration time in T2 phase. (**C**) Exploration time in T3 phase. New indicates the new object; F1, F2, F3, F4, F5 indicate the familiar objects. Data are expressed as mean ± SEM *p<0.05 new object vs all the familiar objects. Duncan *post - hoc* analysis.

5.3.1. Effect of ventral hippocampal lesion on object WM in high memory load conditions

We expanded on the results obtained in the study of Sannino et al. 2012 (Sannino, Russo et al. 2012) investigating the role of the ventral hippocampus in object WM in HML conditions using the 6-DOT. In the T1 we examined the total distance travelled and we did not find significant difference in the total distance travelled between control and ventral hippocampus lesioned mice

(**Figure 5.3.1.A**). Also for the total exploration, we did not find significant difference between the two experimental groups (**Figure 5.3.1.B**). In the T3 phase, both control and ventral hippocampus lesioned mice were able to discriminate the new object compared to the familiar ones. This analysis shows that, unlike the dorsal hippocampus, the ventral hippocampus is not involved in object WM in HML conditions (**Figure 5.3.1.C)**.

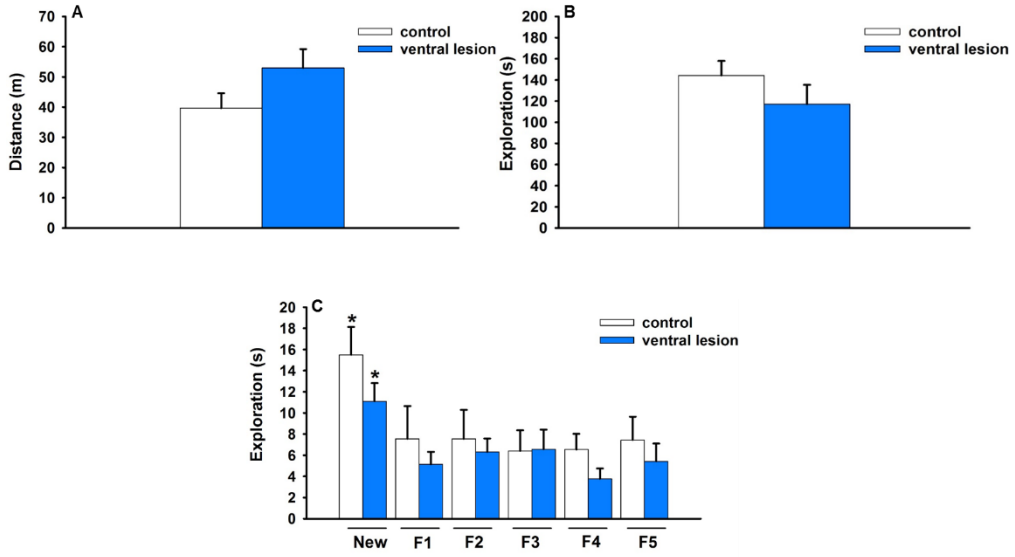


Fig.5.3.1. 6-DOT. (**A**) Total distance travelled in T1 phase in control and ventral hp lesioned mice. (**B**) Exploration time in T2 phase. (**C**) Exploration time in T3 phase. New indicates the new object; F1, F2, F3, F4, F5 indicate the familiar objects. Data are expressed as mean ± SEM *p<0.05 new object vs all the familiar objects. Duncan *post - hoc* analysis.

5.4. Effect of dorsal hippocampus lesion on object WM in low memory load conditions

6- IOT was used as a control test for the 6-DOT, T1 phase was identical to the 6-DOT; in T2 mice had to explore 6 identical objects (overall exploration 35 sec). During the T3 phase, an object was replaced with a new one, and the others with identical copies. This test represented a control test because being the objects identical, the memory load is low. The analysis of the distance travelled in T1 showed no significant difference between the two groups (**Figure 5.4.A**). In addition, the analysis of the total exploration did not show significant difference between control and dorsal hippocampus lesioned mice (**Figure 5.4.B**). Duncan *post - hoc* analysis showed that control and dorsal hippocampus lesioned mice discriminate the new object

compared to the familiar ones. This analysis confirms previous findings showing that the dorsal hippocampus is not involved in object working memory when the load of memory is low (**Figure 5.4.C**).

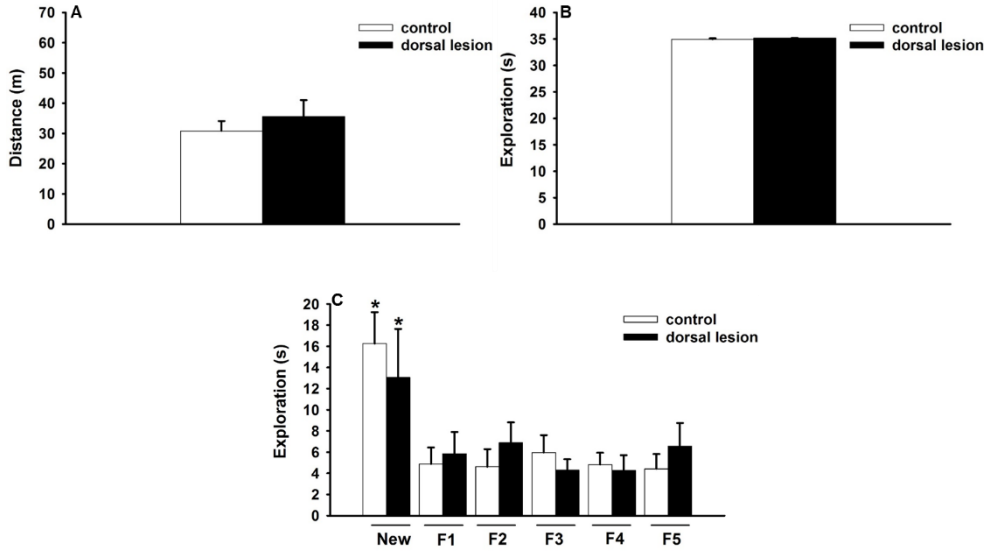


Fig.5.4. 6-IOT. (**A**) Total distance travelled in T1 phase in control and dorsal hp lesioned mice. (**B**) Exploration time in T2 phase. (**C**) Exploration time in T3 phase. New indicates the new object; F1, F2, F3, F4, F5 indicate the familiar objects. Data are expressed as mean ± SEM *p<0.05 new object vs all the familiar objects. Duncan *post - hoc* analysis.

5.4.1. Effect of ventral hippocampus lesion on object WM in low memory load conditions

The analysis of T1 distance revealed no significant differences between the two experimental groups in the distance travelled and also in the total exploration (**Figure 5.4.1. A, B**). In the T3, Duncan *post - hoc* analysis showed that both control and ventral hippocampus lesioned mice were able to discriminate the new object compared to the familiar ones. This suggest that ventral hippocampus, as the dorsal hippocampus, is not involved in object working memory in low memory load conditions (**Figure 5.4.1. C**).

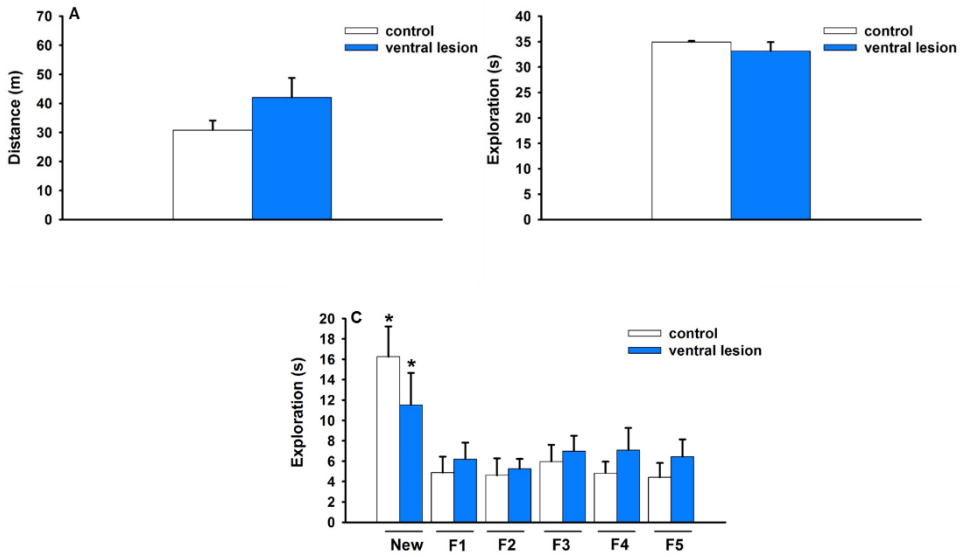


Fig.5.4.1. 6-IOT. (**A**) Total distance travelled in T1 phase in control and ventral hp lesioned mice. (**B**) Exploration time in T2 phase. (**C**) Exploration time in T3 phase. New indicates the new object; F1, F2, F3, F4, F5 indicate the familiar objects. Data are expressed as mean ± SEM *p<0.05 new object vs all the familiar objects. Duncan *post - hoc* analysis.

5.5. Effect of dorsal hippocampus lesion on spatial LTM

Long-term spatial memory was measured by subjecting the animals to the Morris water maze, using a massive procedure. The two- way ANOVA for the latency to reach the platform during the training day showed that dorsal hippocampus lesion increased the latency to reach the platform during training [treatment ($F_{1,20}$=7.295;p=0.0137); sessions ($F_{5,100}$=0.0504); sessions x treatment ($F_{5,100}$=1.183;p=0.3229)]. Duncan *post - hoc* analysis showed that control mice reduced the latency to reach the platform in the two last sessions compared to the first session, while dorsal hippocampus lesioned mice did not show a significant reduction in the latency to reach the platform across training sessions (**Figure 5.5.A**). A similar although, less severe effect was observed with a two-way ANOVA on the distance travelled to reach the platform [treatment ($F_{1,20}$=3.790;p=0.0657); sessions ($F_{5,100}$=3.771;p=0.0036); sessions x treatment ($F_{5,100}$=0.867;p=0.5059)]. Duncan *post - hoc* analysis showed that control mice, but not dorsal hippocampus lesioned mice, reduced the distance to reach the platform in the last session as compared to the first session (**Figure 5.5.B**).

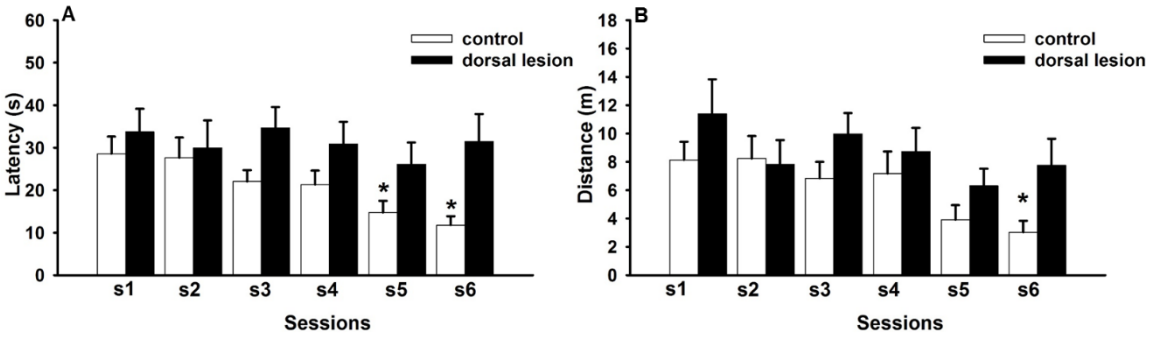


Fig. 5.5. Training phase of the Morris water maze massive test. (**A**) Latency to reach the platform for control and dorsal hp lesioned mice. S1, s2, s3, s4, s5, s6 indicate the sessions. Data are expressed as mean ±SEM. *p<0.05 vs s1, Duncan *post- hoc* analysis. (**B**) Distance travelled to reach the platform. Data are expressed as mean ± SEM. *p <0.05 s6 vs s1. Duncan *post - hoc* analysis.

Relative time permanence in the target quadrant during the probe trial is an index of long-term memory. The two-way ANOVA for repeated measures on the percentage of time in quadrants showed that dorsal hippocampus lesion impaired the preference for the target quadrant [treatment ($F_{1,20}$=0.908;p=0.3521); quadrants ($F_{3,60}$=14.484;p<0.0001); quadrants x treatment ($F_{3,60}$=6.592;p=0.0006)]. Duncan *post - hoc* analysis confirmed that while control mice spent more time in the quadrant in which the platform was located, the dorsal hippocampus lesioned mice randomly searched the platform around all four quadrants (**Figure 5.5.1.A**). This result was further confirmed with the analysis of the entries in the annulus, which gives a more precise indication where the platform was located [treatment ($F_{1,20}$=4.094;p=0.5666); annulus ($F_{3,60}$=9.378;p<0.001); annuli x treatment ($F_{3,60}$=3.490;p=0.0210)]. Duncan *post - hoc* analysis revealed that control mice made more entries in the annulus target while dorsal hippocampus lesioned mice did not remember the precise location of the platform. All together, these analyses indicated that the lesion we performed in the dorsal hippocampus was sufficient to impair spatial LTM (**Figure 5.5.1.B**).

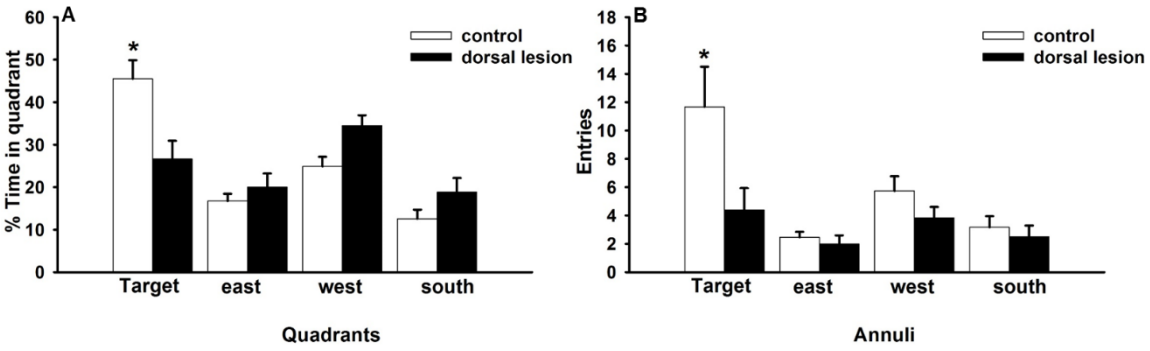


Fig. 5.5.1. Probe phase of the Morris water maze massive test. (**A**) Percentage of the time in quadrant for control and dorsal hp lesioned mice. Data are expressed as mean ± SEM. * p<0.05 target quadrant vs all other quadrants, Duncan *post- hoc* analysis. (**B**) Entries in the annulus. Data are expressed as mean ± SEM. *p<0.05 target annulus vs all other annuli. Duncan *post - hoc* analysis.

5.6. Effect of ventral hippocampus lesion on spatial LTM

Animals with lesion of the ventral hippocampus where much slower [treatment ($F_{1,25}$=9.812; p=0.0044); sessions ($F_{5,125}$=6.428; p<0.0001); sessions x treatment ($F_{5,125}$=0.399; p=0.8486)] and travelled longer distance [treatment ($F_{1,25}$=4.385;p=0.0466); sessions ($F_{5,125}$=11.994;p<0.0001); sessions x treatment ($F_{5,125}$=0.853;p=0.5147)] (**Figure 5.6.A**), as compared to control mice, to find the platform during training sessions. However, Duncan *post - hoc* analysis showed that by the last training sessions ventral hippocampus mice improved performance as compared to the very first training session (**Figure 5.6. A-B)**.

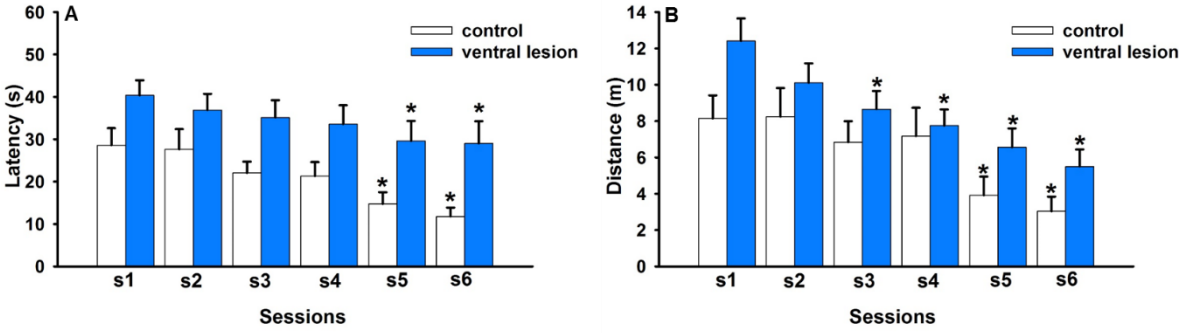


Fig. **5.6. Training phase of the Morris water maze massive test.** (**A**) Latency to reach the platform for control and ventral hp lesioned mice. S1, s2, s3, s4, s5, s6 indicate the sessions. Data are expressed as mean ±SEM. *p<0.05 s5 and s6 vs s1. Duncan *post- hoc* analysis. (**B**) Distance travelled to reach the platform. Data are expressed as mean ± SEM. *p <0.05 vs s1. Duncan *post - hoc* analysis.

The two-way ANOVA for the percentage of time in quadrant showed that, as well as for the dorsal lesioned mice, ventral hippocampus lesioned mice did not show a preference for the correct quadrant [treatment ($F_{1,25}$=1.140;p=0.2958); quadrants ($F_{3,75}$=8.550;p<0.0001); quadrants x treatment ($F_{3,75}$=3.798;p=0.0136)]; Duncan *post - hoc* analysis confirmed that ventral hippocampus lesioned mice did not spent more time in the quadrant in which the platform was located (**Figure 5.6.1.A**). These results were further confirmed by the analysis of the entries in the annulus in which the two-way ANOVA showed once again a lack of preference for the target annulus in the ventral hippocampus lesioned group [treatment ($F_{1,25}$=2.665;p=0.1151); annulus ($F_{3,75}$=12.210;p<0.0001); annuli x treatment ($F_{3,75}$=3.196;p=0.0282)]. Duncan *post - hoc* analysis confirmed that ventral hippocampus lesioned mice did not rememeber the precise location of the platform (**Figure 5.6.1.B**). These data suggest an impairment in ventral hippocampus lesioned mice in spatial LTM as well as dorsal hippocampus lesioned mice.

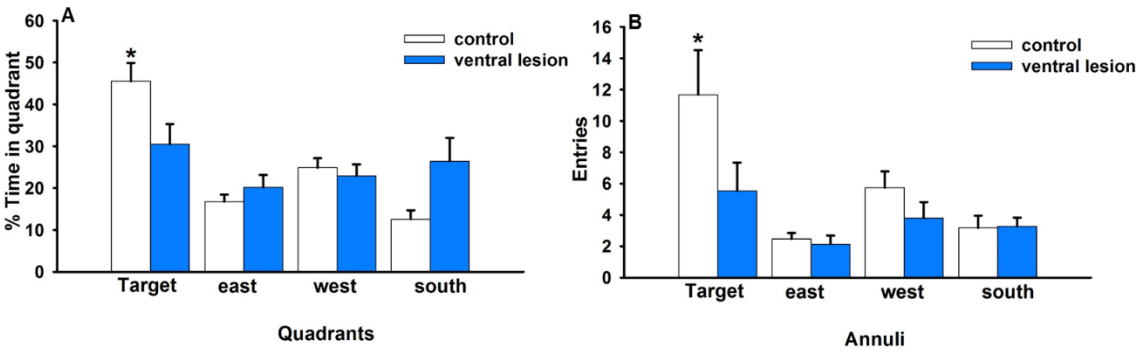


Fig. 5.6.1. Probe phase of the Morris water maze massive test. (**A**) Percentage of the time in quadrant for control and ventral hp lesioned mice. Data are expressed as mean ± SEM. * p<0.05 target quadrant vs all other quadrants. Duncan *post- hoc* analysis. (**B**) Entries in the annulus. Data are expressed as mean ± SEM. *p<0.05 target annulus vs all other annuli. Duncan *post- hoc* analysis.

5.7. Effect of dorsal and ventral lesion in spatial WM

We tested the hypothesis that the dorsal and ventral hippocampus were involved in WML capacity. To this aim, we used mice with a selective lesion of the dorsal hippocampus and the ventral hippocampus. Eight arms radial maze was designed to assess the contribution of the information load on spatial memory. The WM load was increased increasing the number of open/baited arms. During the pre-training phase in the confinement procedure, only two of the eight arms of the radial maze were open and baited. One-way ANOVA for the mean number of

errors showed that there are differences between the three experimental groups [treatment ($F_{2,27}$=3.447; p=0.0464)], Duncan *post - hoc* analysis showed that ventral hippocampus lesioned mice made significatively more errors than dorsal hippocampus lesioned mice (**Figure 5.7.)**, this could be due to the major number of the total entries of the ventral hippocampus lesioned mice indeed the calculation of the number of total entries showed a difference among the three experimental groups [treatment ($F_{2,27}$= 5.454; p=0.0102)]. Duncan *post - hoc* analysis showed that ventral hippocampus lesioned mice made more enteries than control and dorsal hippocampus lesioned mice (data not shown).

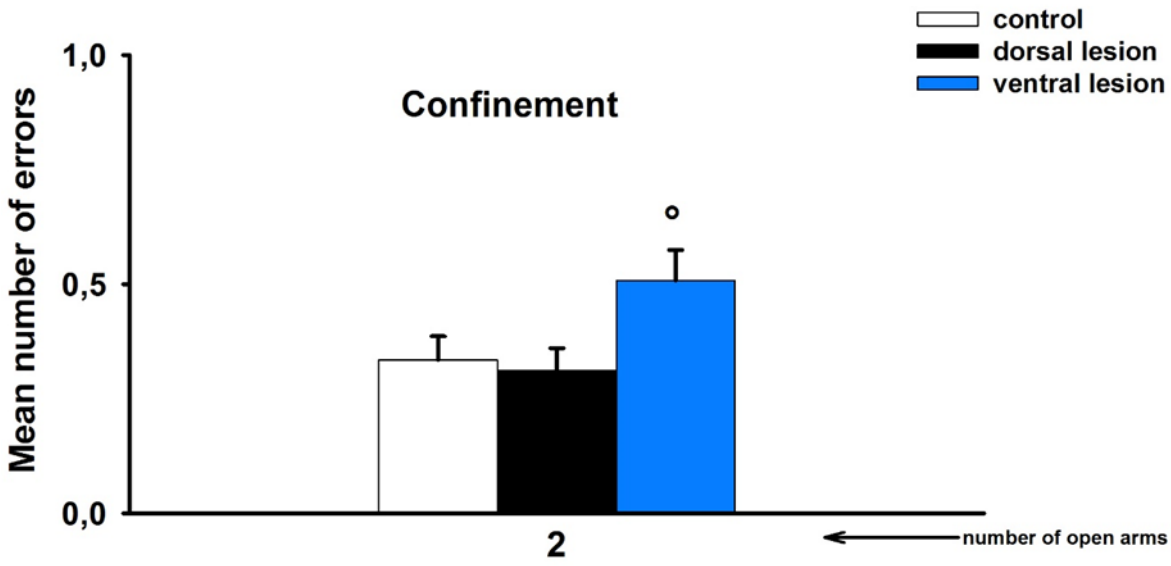


Fig.5.7. Mean number of errors in the pre - training phase phase. Ventral hp lesioned mice make significatively more errors than dorsal hp lesioned mice. Data are expressed as mean ±SEM. ° p<0.05 ventral hp lesioned mice vs dorsal hp lesioned mice. Duncan *post - hoc* analysis.

In the confinement procedure, the number of open/baited arms changed from 3, 6 and 8 between trials and among the training days. We firstly analysed the mean number of errors at 3, 6 and 8 open/baited arms in the five days of confinement and four days of no - confinement procedure for both dorsal and ventral hippocampus lesioned mice. The three-way ANOVA for repeated measures showed that increasing the number of open arms increased the memory load, depending on the procedure (confinement/no - confinement used) [procedure ($F_{1,25}$=92.570;p<0.0001); number of open arms ($F_{2,50}$=142.753;p<0.0001); procedure x number

of open arms ($F_{2,50}$=19.009;p<0.0001)]; hippocampus lesion impaired performance depending on the number of open arms and on the procedure, [treatment ($F_{2,25}$=7.137;p=0.0035); procedure x treatment ($F_{1,25}$=6-552;p=0.0052); number of open arms x treatment ($F_{4,50}$=5.061;p=0.0017)]. No significant effect of the multiple interaction between procedure x number of open arms x treatment ($F_{4, 50}$=0.443; p=0.7771) was observed (**Figure 5.7.1.)**.

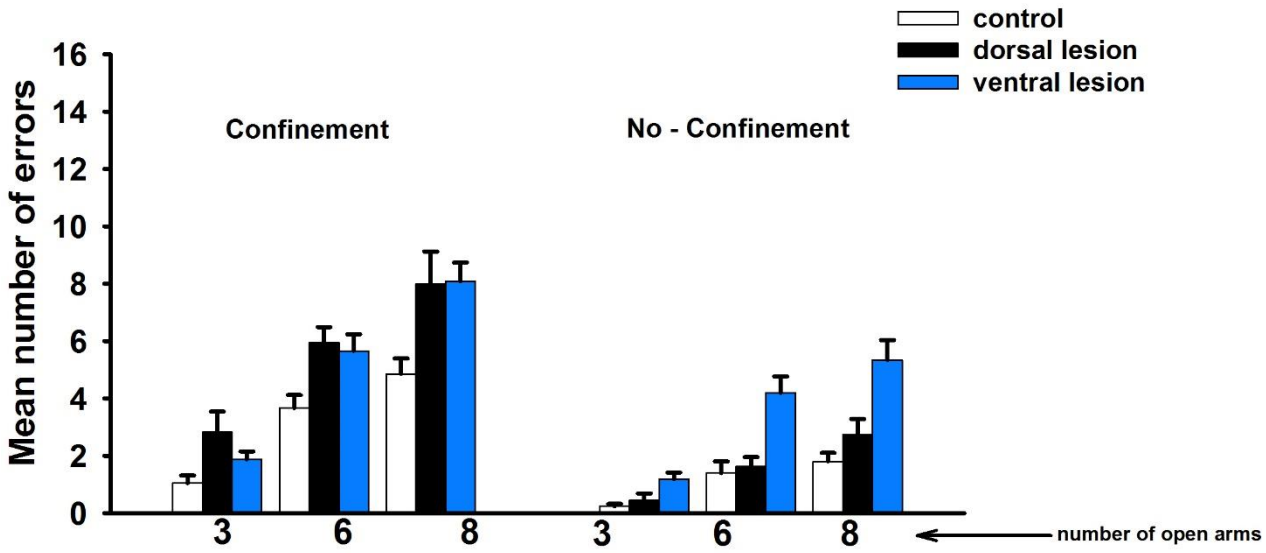


Fig.5.7.1. Mean number of errors of the training phase in the confinement and no - confinement procedure of the working memory capacity radial maze task. Mean number of errors of control, dorsal and ventral hp lesioned mice in the five days of the confinement procedure and in four days of the no - confinement procedure at 3, 6 and 8 open/baited arms. Data are expressed as mean ± SEM.

To dissociate the effects of the dorsal and the ventral hippocampus lesion, we performed a three-way ANOVA for repeated measure for each of the two groups as compared to the control group. Dorsal hippocampus lesion impaired performance depending on the procedure (confinement/ no - confinement used), [treatment ($F_{1, 14}$=5.464; p=0.0348); procedure ($F_{1, 14}$=78.110; p<0.0001); procedure x treatment ($F_{1, 14}$=5.387; p=0.0359)]. Therefore, we have separately analysed the results for each of the two procedures with a two-way ANOVA for repeated measure and found a significant effect of treatment in the confinement procedure [($F_{1, 16}$=9.699; p=0.0067)]. Furthermore, in the confinement procedure we found that increasing the number of open/baited arms also leaded to an increased number of errors [number of open arms ($F_{2, 32}$=69.089;p<0.0001)]; the same result was not observed in the no - confinement procedure, in which the number of errors was not depended on the treatment [(F1,14=1.201;p=0.2916)]. In the

no - confinement procedure, although the number of errors was dramatically reduced as compared to the confinement procedure, the number of open arm leaded to an increase in the number of errors in both groups [number of open arms ($F_{2,28}=38.317$;$p<0.0001$)]. In the no - confinement procedure the ANOVA did not reveal a significant effect for the interaction number of open arms and treatment [number of open arms x treatment ($F_{2,28}=1.755$;$p=0.1914$)], but Duncan *post - hoc* analysis showed that control mice made more errors at 6 and 8 open/baited arms as compared to 3 arms, while dorsal hippocampus lesioned mice made more errors at 8 arms compared to 6 open/baited arms, also revealing that the number of errors increased with the number of open/baited arms. All together, these findings showed that: **1.** By increasing the number of open/baited arms we increased the memory load in the confinement procedure; this effect also much less evident as compared to the confinement procedure, was also evident in the no confinement procedure; **2.** Lesion of the dorsal hippocampus impaired performance in the confinement but not in the no confinement procedure, and this effect was more evident with the highest memory load (**Figure 5.7.2.)**.

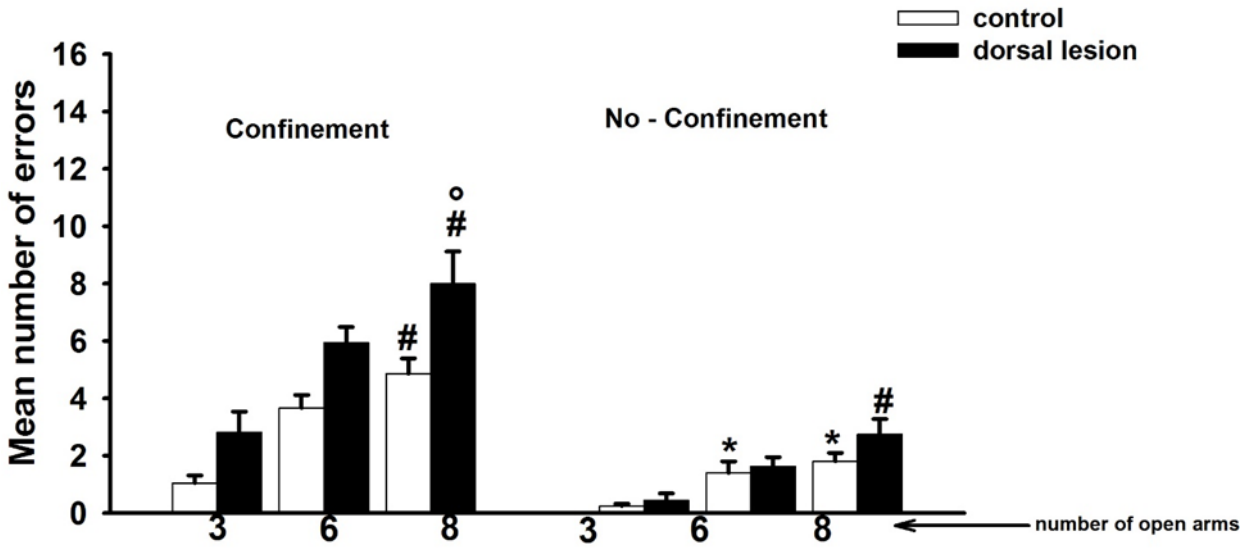


Fig.5.7.2. Mean number of errors of control and dorsal hp lesioned mice in the training phase, in the confinement and no - confinement procedure of the working memory capacity radial maze task. Mean number of errors of control and dorsal hp lesioned mice in the five days of the confinement procedure and in four days of the no - confinement procedure at 3, 6 and 8 open/baited arms. Data are expressed as mean ± SEM. * $p < 0.05$ vs 3 arms, within group, within procedure; # $p < 0.05$ vs 6 arms, within group, within procedure; ° control vs dorsal hp lesioned animals. Duncan *post - hoc* analysis.

The analysis of the mean number of errors at 3, 6 and 8 open/baited arms in the confinement and no - confinement procedure showed that ventral hippocampus lesion impaired performance in

both cases [treatment ($F_{1,18}$=16.191;p=0.0008), procedure ($F_{1,18}$=44.569;p<0.0001), procedure x treatment ($F_{1,18}$=1.354;p=0.2597)], and the effect was dependent on the number of open/baited arms [number of open arms ($F_{2,36}$=95.552;p<0.0001); number of open arms x treatment ($F_{2,36}$=8.165:p=0.0012); procedure x number of open arms ($F_{2,36}$=12.337;p<0.0001), procedure x number of open arms x treatment ($F_{2,36}$=0.416;p=0.6630)]. These results suggest an involvement of the ventral hippocampus in spatial WM in both confinement and no - confinement procedure unlike the dorsal hippocampus lesioned, which is involved in the spatial WM only in the confinement procedure (**Figure 5.7.3.)**. The two-way ANOVA on the confinement procedure showed that ventral hippocampus lesioned impaired performance depending on the number of open/baited arms [treatment ($F_{1,20}$=12.812; p=0.0019), number of open arms ($F_{2,40}$=92.955; p>0.0001), number of open arms x treatment ($F_{2,40}$=5.202; p=0.0098)]. Duncan *post - hoc* analysis showed that ventral hippocampus lesioned mice made more errors than control mice at 8 open/baited arms. In the no - confinement procedure we found an overlapping effect of the ventral hippocampus lesion [treatment (F1, 18=16.678; p=0.0007)], number of open arms [(F2, 36=38.618; p<0.0001)], number of arms x treatment (F2, 36=7.868; p=0.0015)]. Duncan *post - hoc* analysis revealed that ventral hippocampus lesioned mice made significatively more errors at 6 and 8 open/baited arms as compared to control mice. This analysis suggests that the ventral hippocampus lesioned mice are impaired in spatial WM in both confinement and no - confinement procedure unlike dorsal hippocampus lesioned mice, which are impaired in spatial WM only in the confinement procedure (**Figure 5.7.3.**).

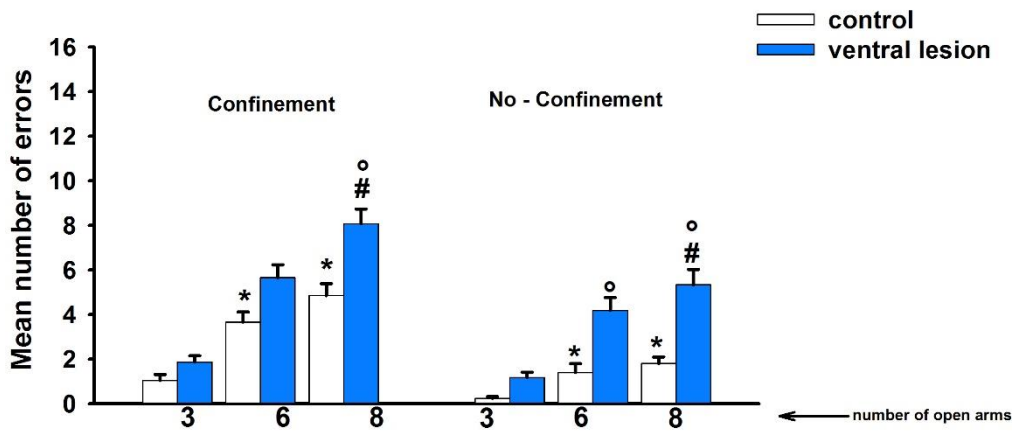
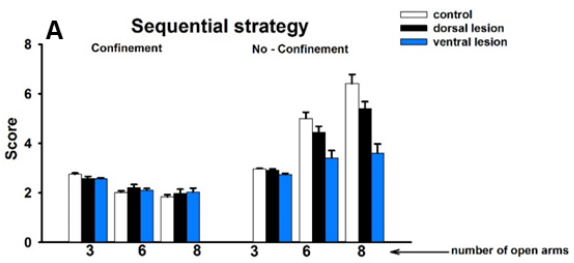


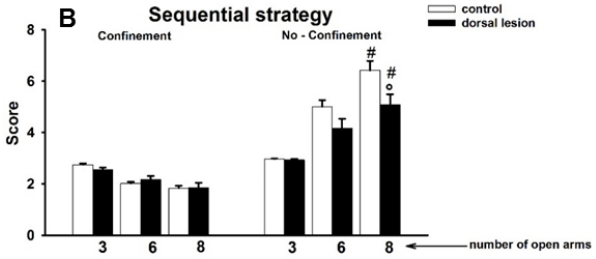
Fig.5.7.3. **Mean number of errors of control and ventral hp lesioned animals in the training phase, in the confinement and no - confinement procedure of the working memory capacity radial maze task.** Mean number of errors in control and ventral hp lesioned mice in the five days of the confinement procedure and in four days of the no - confinement procedure at 3, 6 and 8 open/baited arms. Data are expressed as mean ± SEM. *p < 0.05 vs 3 arms, within group, within procedure; # p < 0.05 vs 6 arms, within group, within procedure; ° p < 0.05 vs control animals, within arm, within procedure. Duncan *post - hoc* analysis.

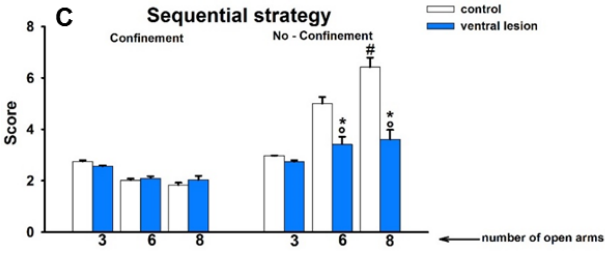
The confinement procedure was introduced to reduce the development of the sequential strategy, which consists in consecutive entering in adjacent arms. The sequential strategy does not require the use of distal visual cues to solve the task, as it can be totally based on egocentric information. Another type of strategy that has been described in the radial maze (Dubreuil, Tixier et al. 2003) is the alternating strategy, which consists in entering in alternating arms. To evaluate the use of the these two strategies in the confinement and no - confinement procedure we calculated a strategy score (see methods) for each of them and analysed the effects of the lesion on their use with a three-way ANOVA for repeated measures. The analysis showed that mice used the sequential strategy depending on the procedure and on the interaction between procedure and the number of open/baited arms [procedure ($F_{1, 25}$=168.851; p<0.0001); number of open arms ($F_{2, 50}$=26.061; p<0.0001); procedure x number of open arms ($F_{2, 50}$=131.325; p<0.0001)]. Hippocampus lesion impaired the use of the sequential strategy depending on the procedure and on the number of open/baited arms [treatment ($F_{2, 25}$=8.601; p=0.0014); procedure x treatment ($F_{2, 25}$=11.758; p=0.0003); number of open arms x treatment ($F_{4, 50}$=5.380; p=0.0011); procedure x number of open arms x treatment ($F_{4, 50}$=12.647; p<0.0001)] (**Figure 5.7.4.A**). Based on these findings showing that during the confinement procedure animals do not rely on the sequential strategy, we focused the analysis on the no - confinement procedure, separately analysing the results for the two hippocampal lesioned groups. The sequential strategy developed differently in the two groups depending on the number of open/baited arms [treatment (F1, 14=5.022; p=0.0421), number of open arms (F2, 28=89.072; p<0.0001), number of open arms x treatment (F2, 28=4.781; p=0.0164)]. Duncan *post - hoc* analysis revealed that both control and dorsal hippocampus lesioned mice used the sequential strategy more when the number of open/baited arms is 6 and 8, as compared to 3 open/baited arms. Dorsal hippocampus mice were impaired in the use of the sequential strategy as compared to control only when animals were confronted with 8 open/baited arms (**Figure 5.7.4.B**). A similar impairment was observed for the ventral hippocampus lesioned animals [treatment (F1, 18=22.262; p=0.0002), number of open arms (F2, 36=59.663; p<0.0001), number of open arms x treatment (F2, 36=20.875; p<0.0001)]. Duncan *post - hoc* analysis showed, however, that ventral hippocampus lesioned mice were impaired in the use of the sequential strategy at both 6 and 8 open/baited arms. This analysis suggests that: **1.** The use of the sequential strategy depends on the procedure; **2.** Hippocampus lesion does not affect the use of the sequential strategy in the confinement procedure; **3.** In the no - confinement procedure the use of the sequential strategy increases with the increase of the number of open/baited arms; **4.** Both the dorsal and the ventral hippocampus lesion impaired the use of the sequential strategy, but the effect was more evident after the ventral lesion in high memory load conditions (**Figure 5.7.4.C)**. As concerning the alternating strategy, a first observation is that we

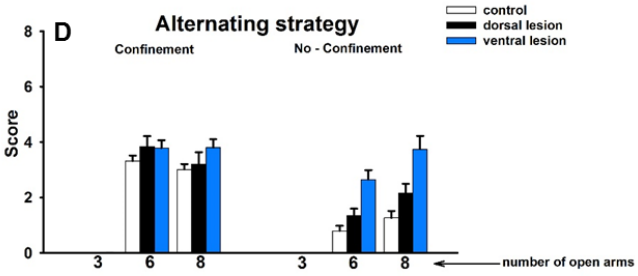
found score 0 when only 3 arms were open/baited. The three-way ANOVA for the score of the alternating strategy for the control, dorsal and ventral hippocampus lesioned mice showed that the three groups differently used the alternating strategy depending on the procedure (confinement/no - confinement used) and on the number of open/baited arms [treatment ($F_{2,25}$=10.561;p=0.0005), procedure ($F_{1,25}$=55.891;p<0.0001), procedure x treatment ($F_{1,25}$=6.069;p=0.0071), number of open arms ($F_{2,50}$=252.415;p<0.0001), number of open arms x treatment ($F_{4,50}$=6.877;p=0.0002), procedure x number of open arms ($F_{2,50}$=22.522;p<0.0001)] (**Figure 5.2.E**) (**Figure 5.7.4.D**). To dissociate the effects of the dorsal and the ventral hippocampus lesion, we performed a three- way ANOVA for repeated measures for each of the two groups as compared to the control group. The three-way ANOVA between control and dorsal hippocampus lesioned mice showed that the use of the alternating strategy was different between the two experimental groups depending on and on the number of open/baited arms [treatment ($F_{1,14}$=5.582;p=0.0332); procedure ($F_{1,14}$=66.802;p<0.0001), number of open arms ($F_{2,28}$=198.911;p<0.0001); procedure x number of open arms ($F_{2,28}$=22.745;p<0.0001)]. This effect was due to differences in the use of alternating strategy by the two experimental groups whose use also depended on the number of open/baited arms [treatment ($F_{1, 14}$=4.485; p=0.0450); number of open arms ($F_{2, 28}$=42.479; p<0.0001); number of open arms x treatment ($F_{2, 28}$=2.890; p=0.0723)]. Duncan *post - hoc* analysis showed that dorsal hippocampus lesioned mice used the alternating strategy more than control mice, and this effect was evidenced by a significant increase in the score when switching to 6 and 8 open/baited arms (**Figure 5.7.4.E**). We found different results analysing the score of the alternating strategy for control and ventral hippocampus lesioned mice, indeed the three-way ANOVA showed that the use of the strategy was different between the two experimental groups depending on the procedure and on the number of open/baited arms [treatment ($F_{1,18}$=16.924;p=0.0007); procedure ($F_{1,18}$=38.664;p<0.0001); procedure x treatment ($F_{1,18}$=12.336;p=0.0025); number of open arms ($F_{2,36}$=168.308;p<0.0001); number of open arms x treatment ($F_{2,36}$=11.498;p=0.0001); procedure x number of open arms ($F_{2,36}$=14.676;p<0.0001); procedure x number of open arms x treatment ($F_{2,36}$=3.395;p=0.0446)]. In the confinement procedure animals with ventral hippocampus lesion used the alternating strategy more than the control group, independently on the number of open /baited arms [treatment ($F_{1, 20}$=6.657; p=0.0179); number of open arms ($F_{2, 40}$=164.653; p<0.0001); number of open arms x treatment ($F_{2, 40}$=1.586; p=0.2173)]. In the no - confinment procedure the increase in the use of the alternating strategy as compared to the control group was better evidenced [treatment ($F_{1, 18}$=18.752; p<0.0001); number of open arms ($F_{2, 36}$=51.160; p<0.0001); number of open arms x treatment ($F_{2, 36}$=12.999; p<0.0001)]. Duncan *post - hoc* analysis showed that ventral hippocampus lesioned mice used more the alternating strategy than

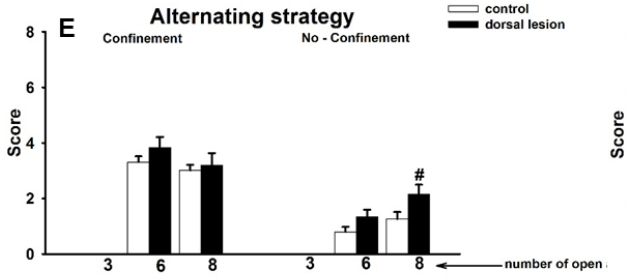
control mice at both 6 and 8 open/baited arms. All together these data suggest that: **1.** The alternating strategy is predominatly used by control mice during the confinement procedure, and much less during the no - confinement procedure; **2.** The use of alternating strategy is not depedent on the number of arms open/baited; **3.** Ventral hippocampus lesion increases the use of the alternating strategy; this effect was much more evident in the no - confinement procedure and with an high number of open/baited arms (**Figure 5.7.4.F**).


A
Sequential strategy
Confinement
No - Confinement
control
dorsal lesion
ventral lesion
Score
number of open arms


B
Sequential strategy
Confinement
No - Confinement
control
dorsal lesion
Score
number of open arms


C
Sequential strategy
Confinement
No - Confinement
control
ventral lesion
Score
number of open arms


D
Alternating strategy
Confinement
No - Confinement
control
dorsal lesion
ventral lesion
Score
number of open arms


E
Alternating strategy
Confinement
No - Confinement
control
dorsal lesion
Score

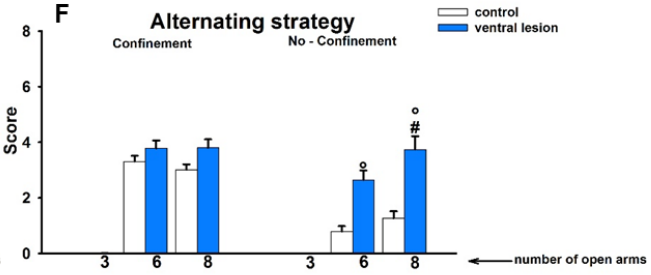

F
Alternating strategy
Confinement
No - Confinement
control
ventral lesion
Score
number of open arms

Fig. 5.7.4. Sequential and alternating strategies in the training phase in the confinement / no – confinement procedure of the working memory capacity radial maze task.

(**A**) Score of the sequential strategy in control, dorsal and ventral hp lesioned mice in the confinement/no – confinement procedure at 3, 6 and 8 open/baited arms. (**B**) Score of the sequential strategy for control and dorsal hp lesioned mice in the confinement/no – confinement procedure at 3, 6 and 8 open/baited arms. # p<0.05 8 vs 6 open/baited arms within group, within procedure; ° p <0.05 dorsal hp lesioned mice vs control, Duncan *post – hoc* analysis. (**C**) Score of the sequential strategy for control and ventral hp lesioned mice in the confinement/no – confinement procedure at 3, 6 and 8 open/baited arms. * p<0.05 8 and 6 vs 3 open/baited arms within group, within procedure; ° p<0.05 ventral hp lesioned mice vs control, Duncan *post – hoc* analysis. (**D**) Score of the alternating strategy for control, dorsal and ventral hp lesioned mice in the confinement/no – confinement procedure at 3, 6 and 8 open/baited arms. Data are expressed as mean ± SEM. (**E**) Score of the alternating strategy for control and dorsal hp lesioned mice in the confinement/no - confinement procedure at 3, 6 and 8 open/baited arms. #p<0.05 8 vs 6 open/baited arms within group, within procedure. Duncan *post – hoc* analysis. (**F**) Score of the alternating strategy for control and ventral hp lesioned mice in the confinement/no – confinement procedure at 3, 6 and 8 open/baited arms. # p<0.05 8 vs 6 open/baited arms within group, within procedure; °p <0.05 ventral hp lesioned mice vs control. Duncan *post – hoc* analysis.

6. Discussion

Traditionally WM was associated with dopaminergic fronto - striatal network, but recent evidence shows that the hippocampus has a role in WM in HML conditions. The WMC refers to the amount of the information that one can retain for a short period (from sec to minutes). The aim of this work was to study the role of dorsal and ventral hippocampus in WMC in CD1 mice using a neurotoxic selective dorsal and ventral hippocampal lesion approach. To study spatial WMC, we tested control and lesioned mice in a WMC version of the eight arms radial maze task; when the use of egocentric strategies was prevented by the use of a confinement procedure, both lesioned groups were impaired in WMC in HML conditions. Removal of confinement allowed control mice to switch to the use of the sequential strategy, which lowered the memory load, and consequently the number of errors independently on the number of arms opened/baited. In this condition, lesion of the ventral, but not of the dorsal hippocampus, impaired performance as it impaired the use of the sequential strategy. In conclusion, our results suggest a complementary role of the dorsal and ventral hippocampus in mediating allocentric spatial WMC, and a dissociation between the two subregions in mediating egocentric WMC and object WMC. Our data suggest that the dorsal and the ventral hippocampus regulate WMC by processing allocentric and egocentric spatial information, respectively. The ventral hippocampus is more involved in mediating the acquisition of egocentric strategies to solve the task. In contrast, only the dorsal part regulates WMC for objects.

6.1. Role of the dorsal and ventral hippocampus in anxiety

Ventral hippocampus is defined as a "hot hippocampus"linked to emotion and to responses to stress and whose dysfunction leads to affective disorder as depression (Fanselow and Dong 2010). Here we performed the elevated plus maze, a test widely used in literature to assess the effect of dorsal and ventral lesion on anxiety. Our results showed that the dorsal hippocampus lesion did not affect behavior in this task; this result is in agreement with studies in literature showing that dorsal hippocampus through its connection with the enthorinal cortex, receives information from the visual, auditory and somatosensorial cortices (Moser and Moser 1998), and it is more involved in spatial learning, than in emotional processing. In line with previous lesion studies in rats using the same task, ventral hippocampus lesioned animals spend more time in the open arms, as animals receiving an anxiolytic drugs (Kjelstrup, Tuvnes et al. 2002). The ventral hippocampus is connected with amygdalar nuclei essential components of the Pavlovian fear conditioning and with caudate-medial (shell) nucleus accumbens, which has an important role in motivation and reward processing and hypothalamus (Fanselow and Dong 2010). Due to the nature of its connection, here we confirm that the ventral hippocampus is more involved in emotional processing.

6.2. Role of the dorsal and ventral hippocampus in object WM capacity

Recognition memory is defined as the capacity to recognize a previously encountered item as familiar and depends on the integrity of the medial temporal lobe (Squire et al, 2007). One of the most common task used to test object recognition memory is the NOR. When animals are exposed to a novel and a familiar object they spent more time exploring the novel object than the familiar one. Damage limited to the hippocampus are sufficient to produce an impairment in recognition memory in humans (Squire, Wixted et al. 2007), while in the rat there is less agreement about the involvement of the hippocampus, because recognition memory impairments could depend on the lesion size and on the length of the retention delay used in the tasks (Broadbent, Squire et al. 2004). Beason-Held (Beason-Held, Rosene et al. 1999) demonstrated that ibotenate hippocampal lesion in monkeys impaired both the delayed nonmatching to sample task performance and the delayed recognition span task, in which is required to the animals to identify a novel object in an increasing array of previously presented familiar stimuli. In a previous study we have modified the NOR to study object memory capacity, by increasing the number of different objects the animals had to explore during the study phase; using this task we

have previously showed that CD1 naïve male mice can discriminate up to 6 different objects (in the 6-DOT), and that selective lesion of the dorsal hippocampus reduced the memory capacity from 6 to 4 (Sannino, Russo et al. 2012). Using this task we have replicated these findings and expanded on them by showing that lesion of the ventral hippocampus does not affect memory capacity at 1 min delay. This can suggest that ventral hippocampus lesioned mice could use their intact dorsal hippocampus region to solve the task. Thus, we can conclude that not all the hippocampus is involved in object working memory capacity load, but only its dorsal region (Sannino, Russo et al. 2012).

6.3. Role of the dorsal and ventral hippocampus in spatial LTM

Morris water maze was performed to verify if the lesion in the dorsal and ventral hippocampus resulted in a functional damage in the same hippocampal areas classically studied in spatial LTM test (Moser, Moser et al. 1993, Moser 1995). Morris water maze is classically used to test spatial LTM in rodents (Morris 1984). In our study, we used a modified version of the protocol of the Morris water maze previously described (Ferretti, Sargolini et al. 2007) in which mice were trained with a massive training of four sessions of three trials per session in one single day. In our protocol, mice performed six sessions of three trials and the next day they performed the probe test. Our data show both lesioned groups were impaired in the acquisition of the task. In particular, dorsal hippocampus lesioned mice were impaired during both training and testing where they did not remember neither the target quadrant nor the precise location of the platform. These results are in agreement with previous finding using the same or a different procedures (Moser, Moser et al. 1993). Ventral hippocampus lesioned mice were also impaired, but the deficit was less severe as they reduced the latency to reach the platform in the two last sessions compared to the very first session. However, in the probe test ventral hippocampus lesioned mice as well as dorsal hippocampus lesioned mice did not remember neither the quadrant in which the platform is located nor the precise localization of the platform as indicated in the entries in the annulus. Our data suggest an involvement of both dorsal and ventral hippocampus in mediating spatial LTM. While the role of the dorsal hippocampus in spatial LTM has been consistently reported, studies on the contribute of the ventral portion to this type of memory has given conflicting results. Factors as the amount of training and the extend of the lesion have been suggested to modulate the effects of ventral hippocampus lesion on spatial memory in the water maze. Distributed training across 8 days could attenuate the difference between dorsal and ventral hippocampus lesion (de Hoz, Knox et al. 2003). Furthermore, lesion in the ventral

hippocampus major to the 30-50% of the total hippocampal volume are necessary to induce a learning deficit in the water maze (Moser, Moser et al. 1993). Taken together our results are consistent with these findings showing that extended lesion of the ventral hippocampus (more than 50% of the total hippocampus) impair water maze performance in a massive procedure.

6.4. Role of the dorsal and ventral hippocampus in spatial WM capacity

The eight arms radial maze designed by Olton and Samuelson in 1976 was classically used to test spatial memory in rodents. In their original experiments, rats were placed in the center of the radial maze from where they had to retrieve food placed at the end of every arms. They observed that rats quickly learned to retrieve food from every arm usually entering in seven of eight arms before entering a previously visited arm. They evaluated the percentage of correct response (entering in arms before re - entering in an arm previously visited); this represented the first approach to study spatial WM in rodents. In our study we introduced a new version of the radial maze (Olivito, 2016), in which the WM load was increased by increasing the number of open and baited arms, from 3 (low memory load condition), to 6 (intermediate memory load condition) and 8 (high memory load condition) in order to evaluate spatial WMC. As well as in the previous study, in which we have used inbred C57BL/J mice (Olivito, 2016), control animals increased the number of errors when the number of baited/open arms was increased, suggesting that this behavioral procedure can be used to tap WMC. Previous findings suggesting that to solve the task mice can use the visual cues present in the environment, thus creating a spatial map (Tolman 1948), which establishes a relation between the localization of the reward and the spatial stimuli (allocentric strategy). In addition, the importance of the allocentric strategy was defined in a study by Dudchendko in 1997 (Dudchenko, Goodridge et al. 1997), who trained rats in an eight arms radial maze, dividing them in three groups “clear” in which rats were brought into the maze in a clear container which allow them to see the external environment, an“opaque group” in which rats were brougth into the maze in an opaque container which did not allow them to see the external environemnt and a third group called “opaque + disorientating” in which rats were brought to the apparatus in an opaque container and also were disorientated moving the box in which they were contained. This study showed that rats in the clear group performed better because they could see the cue in the room which were in the same fixed location relative to the reward arms creating a spatial map (Dudchenko, Goodridge et al. 1997). But mice can solve the task also using a simple egocentric sequential strategy (a response learning based on

stimulus - response associations), as demonstrated by Olton in 1977 (Olton 1977). Thus, to prevent the development of the sequential strategy, we introduced a confinement procedure first introduced by Olton et al in 1977 (Olton 1977) and then studied by Dubreuil in 2003 (Dubreuil, Tixier et al. 2003). We confined mice in the center of the apparatus for 5 sec between arm choices. This procedure efficiently prevented the use of the sequential strategy in control mice. It must be said, however, that this procedure also increased the delay, as previously argued by Dubrueil (Dubreuil, Tixier et al. 2003). Although, this difference in the delay might account for some of the differences between the confinement and the no-confinment procedures, it does likely not account for the difference in the use of egocentric strategy. Indeed, in a previous study (Dubreuil, Tixier et al. 2003) using three different delays of confinement, 0, 5 and 10 sec, it was shown that even a confinement of 0 sec was sufficient to reduce the use of the sequential strategy. During the confinement procedure control animals used the alternating strategy. In our study we found that in the confinement procedure the number of errors was memory load-dependent in all three experimental groups; the impairment induced by hippocampal lesion was also memory load dependent. Both subregions are involved in spatial WM, but the effect is memory load dependent as it was significant only when all 8 arms were open. When animals were allowed to switch to the no - confinment procedure the number of errors dramatically dropped, likely due to the use of the sequential strategy. When switching to the no - confinement procedure mice with dorsal hippocampus lesion reduced the number of errors as well as control animals. In contrast, ventral hippocampus lesioned mice were impaired also in the confinement procedure. These data suggested that the ventral hippocampus is involved in the acquisition of the sequential strategy; accordingly, previous findings showed *c-fos* activation in the ventral CA1 subfield in animals trained to use an egocentric strategy in a starmaze task (Fouquet, Babayan et al. 2013). In addition, electrophysiological studies suggested that in ventral hippocampus cells respond to nonspatial information (Royer, Sirota et al. 2010), and ventral CA3 cells are more sensitive to locate maze cues than extramaze landmarks (Olton 1979, Thompson and Best 1989), and that the nonspatial factors that affect the firing of CA3 ventral hippocampus cells are more strongly correlated by the reward and emotional features (Royer, 2010). It has also been demonstrated that ventral hippocampus has a role in temporal order memory, the capacity to distinguish between two spatial localization visited at different points in the time (Wong, Howland et al. 2007). This could also explain the major role of the ventral hippocampus in the sequential strategy, which requires remembering a succession of actions.We therefore asked wether ventral hippocampus lesioned mice relyed on a different spatial strategy to solve the task, and analized the use of the alternating strategy. Alternating strategy which is defined in Dubreuil in 2003 (Dubreuil, Tixier et al. 2003) as entering in two arms separated by

one arm. He found that mice confined for 0 sec increased the use of alternating strategy compared to sequential one. Alternating strategy was predomintaly used by control and dorsal hippocampus lesioned mice during the confinement procedure, with 6 and 8 arms, and prontly abandoned in the no – confinement procedure when they could switch to the sequential one. This strategy shift was not observed in ventral hippocampus lesioned mice which continued to use the alternating strategy also in the no – confinement procedure. This finding suggests that the impairment in the use of the sequential strategy might be due to either an impairment in egocentric WM, but also in behavioral switching: switching from alternating to sequential depending on the task demand. To address this question we are testing another experimental group in the no - confinement not preceded by the confinement procedure. Finally, our results suggest that both dorsal and ventral hippocampus are involved in spatial WM in HML conditions with dorsal hippocampus selectively involved in allocentric spatial WM, and the ventral hippocampus involved in both allocentric and egocentric spatial WM.

7. General conclusions

The data we presented in this study are important in elucidating the distinct role of dorsal and ventral hippocampus in working memory capacity. Our data suggest that both dorsal and ventral hippocampus are impaired in spatial WM. Previous findings reported that the hippocampus is involved in spatial WM in the radial maze, even when a single arm is open/baited. This is not consistent with our findings showing a memory capacity-dependent role of both subregions. This difference might be due to the fact that in these previous study a total hippocampal damage was performed, that might have had additive negative effects on the performance (Dubreuil, Tixier et al. 2003). A similar overlapping role between the two subregions is observed for spatial LTM. These behavioral findings are also consistent with electrophysiological evidence (Poucet, Thinus-Blanc, 1994) showing that place cells are also present in the ventral hippocampus, and their positional firing patterns are characterised by place fields, as the dorsal place cells. All together, these findings suggest that the hippocampus could act as a unitary structure along its septo-temporal axis in processing allocentric spatial information, and that in condition of low memory load a spearing of one of the subregions can compensate for the dysfunction of the other part. In contrast, in conditions of high memory load the whole hippocampus is recruited in the task. Our study confirm previous findings suggesting an interesting dissociation between the two subregions in processing egocentric spatial information; our findings expand on these previous evidence showing that the impairment in the use of the sequential strategy is memory load

dependent. This suggests that the ventral hippocampus might be recruited into egocentric tasks only in condition of long sequences of actions. Electrophysiological studies suggested that in ventral hippocampus cells respond to nonspatial information (Royer, Sirota et al. 2010), and ventral CA3 cells are more sensitive to locate maze cues than extramaze landmarks (Olton 1979, Thompson and Best 1989), and that the nonspatial factors that affect the firing of CA3 ventral hippocampus cells are more strongly correlated by the reward and emotional features (Royer, 2010. The role of the ventral hippocampus in processing action-related egocentric information is in line with its connections with the limbic system, and its role in modulating emotional memory and behavior. The ventral hippocampus projects to the striatum, and through this pathway, it might control action selection. In contrast, only the dorsal part regulates WMC for objects (Sannino, Russo et al. 2012). WM impairment are at the base of several human disorders as well as schizophrenia and schizophrenia – spectrum disorders or autism spectrum disorders. Understanding the neural mechanisms at the base of WM and WMC could be important in contributing to the investigation of these kind of cognitive human deficits.

8. Bibliography

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