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# Long term exposure to combination paradigm of environmental enrichment, physical exercise and diet reverses the spatial memory deficits and restores hippocampal neurogenesis in ventral subicular lesioned rats

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A B S T R A C T

Subiculum is an important structure of the hippocampal formation and plays an imperative role in spatial learning and memory functions. We have demonstrated earlier the cognitive impairment following bilateral ventral subicular lesion (VSL) in rats. We found that short term exposure to enriched environment (EE) did not help to reverse the spatial memory deficits in water maze task suggesting the need for an appropriate enriched paradigm towards the recovery of spatial memory. In the present study, the efficacy of long term exposure of VSL rats to combination paradigm of environmental enrichment (EE), physical exercise and 18 C.W. diet (Combination Therapy – CT) in reversing the spatial memory deficits in Morris water maze task has been studied.

Ibotenate lesioning of ventral subiculum produced significant impairment of performance in the Morris water maze and reduced the hippocampal neurogenesis in rats. Post lesion exposure to C.T. restored the hippocampal neurogenesis and improved the spatial memory functions in VSL rats. Our study supports the hypothesis that the combination paradigm is critical towards the development of an enhanced behavioral and cognitive experience especially in conditions of CNS insults and the associated cognitive dysfunctions.





## 1. Introduction

Hippocampus based spatial memory processing has been studied extensively over a century. Initial studies emphasize mainly on the role of hippocampus, particularly the CA3, CA1 areas in spatial map development. As the spatial map development is a highly complex process, recent studies highlight the importance of each structure of the hippocampal formation in spatial map development. The structures of hippocampal formation – the dentate gyrus, CA1 and CA3 areas of hippocampus, subiculum and entorhinal cortex are connected both anatomically and functionally and hence are integral part of spatial information processing and cognitive map development (Amaral & Witter, 1995; O'Keefe, 1979; O'Mara, 1995, 2005; Brotons-Mas, O'Mara, & Sanchez-Vives, 2006; O'Mara, Sanchez-Vives, Brotons-Mas, & O'Hare, 2009). Subiculum is the least explored structure and needs more attention to elucidate its functional implications in spatial information processing.

Anatomically subiculum occupies a strategic position between hippocampus and entorhinal cortex. It receives dense projections from the CA1 subfield of the hippocampus (Amaral, Dolorfo, & Alvarez-Royo, 1991; Cenquizca & Swanson, 2007; Fuentealba et al., 2008; O'Mara, Commins, Anderson, & Gigg, 2001; O'Mara et al., 2009) and has topographically organized reciprocal connections with the entorhinal cortex (Kloosterman, van Haeften, & Lopes da Silva, 2004; Naber, Lopes da Silva, & Witter, 2001). Subiculum is the major output center of the hippocampal circuit to many cortical and sub-cortical structures; prefrontal cortex, amygdala, nucleus accumbens and hypothalamus (Burwell &

*Abbreviations:* 18 C.W. diet, 18 casein–wheat diet; AD, Alzheimer's disease; CA, Cornu Ammonis; CT, Combination Therapy (combination paradigm of environmental enrichment, physical exercise and 18 C.W. diet); DCX, doublecortin; DCX-IR, doublecortin immuno reactive cells; EE, environmental enrichment; NC, normal control; NC-SH, normal control-standard housing; NC-CT, normal control-combination therapy; VC, vehicle control; VC-SH, vehicle control-standard housing; VSL, ventral subicular lesion; VSL-CT, ventral subicular lesion-combination therapy; VSL-SH, ventral subicular lesion-standard housing.

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Amaral, 1998; Lavenex & Amaral, 2000; Witter, Wouterlood, Naber, & Van Haeften, 2000). Subiculum with its extensive connections is thought to mediate a whole range of neuro-cognitive functions – spatial learning and memory, motivation, etc. (O'Mara et al., 2009). Subiculum receives inputs directly from peri and post rhinal cortices and is involved in encoding and consolidation of spatial information (Burwell & Amaral, 1998; Naber, Witter, & Lopes Silva, 2000) besides consolidation and transfer of previously processed information obtained from CA3/CA1 subfields (Lavenex & Amaral, 2000; Naber et al., 2000). Like hippocampal place cells, subicular cells show characteristic spatially selective firing properties associated with spatial map development (Kim, Ganguli, & Frank, 2012; O'Mara et al., 2009; Sharp, 1997, 2006). Subiculum with a well defined anatomical connections, codes space complementing both hippocampal inputs as well as distinctly update their own firing properties with regard to the position of animal in the environment (O'Mara et al., 2009). Subiculum along with entorhinal cortex would help hippocampus to generate a fine representation of each environment (Hafting, Fyhn, Molden, Moser, & Moser, 2005; Kim et al., 2012; Sharp, 2006). These structures thus play a differential but complementary role in spatial learning and memory functions (Deadwyler & Hampson, 2004; Fanselow & Dong, 2010).

The present study is aimed to understand the role of ventral subicular lesion (VSL) on spatial memory and on adult hippocampal neurogenesis. In addition the study tries to elicit the efficacy of combination paradigms of environmental enrichment and physical exercise together with nutritional supplementation on spatial memory recovery in Morris water maze task as well as on hippocampal neurogenesis in ventral subicular lesioned rats. Hippocampal based memory deficits as well as neurodegeneration of hippocampal structures including the subiculum is of great concern in neurodegenerative diseases such as Alzheimer's disease (Dickson et al., 1992; Wang, Tanila, Puoliväli, Kadish, & van Groen, 2003). Such approaches would help developing appropriate rehabilitation strategies in improving cognitive functions in Alzheimer's disease. We have reported the behavioral recovery in eight arm radial maze tasks but not in Morris water maze tasks following short term exposure to environmental enrichment alone in VSL rats (Bindu, Rekha, & Kutty, 2005). Though both these maze procedures help to assess the spatial abilities, both operates upon different aspects of search strategies using spatial and non spatial cues towards the development of spatial maps. Thus the recovery may be task dependent and hence requires appropriate enrichment programs. Combination paradigms of environmental enrichment, physical exercise and nutritional supplementation has been reported to augment various aspects of neural plasticity events (Fabel et al., 2009; Kempermann, 2012; Pang & Hannan, 2013), provide better utilization of spatial or non spatial cues and strengthen various domains of cognitions leading to behavioral recovery in Morris water maze tasks (Will & Kelche, 1992). The study will help us understand the important role played by subiculum in spatial memory and neurogenesis as well as the feasibility of long term exposure to combination paradigm of EE with physical exercise and diet supplementation in establishing both spatial functions in Morris water maze and hippocampal neurogenesis in VSL rats.

## 2. Materials and methods

### 2.1. Experimental animals

Two months old adult male Wistar rats were used for the study. All experiments were carried out in accordance with the guidelines of Central Animal Research Facility (CARF), at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. Experimental protocol was approved from the institutional animal ethics committee (IAEC) (AEC/45/280/N.P) and care was taken to minimize the pain or discomfort during surgery to the animals.

A total of 119 adult male Wistar rats were used for the present study. Rats (3 rats/cage) were housed in standard polypropylene cages of dimensions 22.5 × 35.5 × 15 cm, provided with ad libitum food (laboratory chow, Amruth Feeds, Pune) and water and maintained on a 12:12 h light/dark cycle. Rats were randomly divided into the following groups i.e. normal control (NC, $n = 46$) group, reared in their home cages without any surgical interventions, vehicle control (VC, $n = 25$) group subjected to bilateral infusion of phosphate buffered saline (0.1 M PBS, pH 7.4) into ventral subiculum and ventral subicular lesioned (VSL, $n = 48$) group, received bilateral infusion of ibotenic acid stereotaxically into the ventral subiculum.

### 2.2. Chemical lesioning of ventral subiculum

Rats from the VSL group ($n = 48$) were anesthetized with the combination of ketamine (80 mg/ kg body weight) and xylazine (10 mg/kg body weight). In addition, xylocaine (2%) was injected subcutaneously over the scalp before the surgery. Flat skull coordinates for ventral subiculum (antero-posterior: −6.7 mm; medio-lateral: 5 mm; dorso-ventral: 7.3 mm) were taken from Paxinos and Watson's rat atlas (2004). Rats were stereotaxically (David Kopf Stereotaxic instrument, USA) injected with Ibotenic acid (Tocris), 2 μg/μL at a rate of 0.2 μL/min/site bilaterally into the ventral subiculum (VS) using 5 μL Hamilton syringe fitted to the micro injector (Harvard instruments, USA). Cannula was left at the infusion site for additional 5 min to avoid the backflow. Rats were allowed to recover post surgery for 10 days before the start of experiments. The vehicle control group (VC; $n = 25$) received same volume of 0.1 M PBS into the ventral subiculum bilaterally.

### 2.3. Spatial Navigation in morris water maze task-phase – I training (acquisition)

A total of 84 rats (NC, $n = 28$, VC, $n = 25$, VSL, $n = 31$) were used for behavioral assessment of spatial navigation in Morris water maze task. The Morris water maze consists of a large metal circular tank with dimensions of 2 m diameter and 3 feet depth (diameter 168 cm, height 60 cm) filled with water (temperature 24 ± 1 °C) to about 30 cm depth, made opaque by adding milk. An escape platform, 10 cm in diameter was kept 1 cm below the water surface in the north east quadrant. The Morris water maze task was carried out in accordance with our earlier study (Bindu et al., 2005).

During habituation (only two trials), the rats (from different groups) were allowed to swim for 90 s with no escape platform. During acquisition, the rats were trained in water maze to locate the hidden platform using reference memory procedure by placing the hidden platform exclusively in the north east quadrant. During training, rats were allowed to swim until they reach the escape platform or for a maximum duration of 90 s. If the rats could not reach the escape platform within 90 s, they were gently guided to the platform. Such guidance was provided only for first two days of training. In each day, during training, rats were released from the North West quadrant in the first trial and from the south west quadrant in the second trial. Latency and swim path was recorded using an automated video tracking system (Columbus Videomex-V). The training session included two trials per day with an inter trial interval of 30 min and the average of two trials is considered for the escape latency. The training continued till the rats learned to reach the hidden platform within 20 s duration following the release into the pool. Twenty-four hours following acquisition of the task, a probe test was performed during which he rats were



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