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Research report

Changes of hippocampal beta-alanine and citrulline levels are paralleling early and late phase of retrieval in the Morris Water Maze

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HIGHLIGHTS

- Hippocampal beta alanine is paralleling spatial memory retrieval in the MWM.
- Hippocampal citrulline is paralleling spatial memory retrieval in the MWM.
- Hippocampal amino acids level changes in late phase of spatial memory retrieval.
- Changes of amino acids metabolism during spatial memory retrieval in the MWM.

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ABSTRACT

Although a series of amino acids (AA) have been associated with spatial memory formation, there is limited information on concentrations of beta-alanine and citrulline in rodent brains. Given the importance of AA metabolism in cognitive functions it was the aim of the study to determine hippocampal levels of beta-alanine and citrulline in rats during two different phases of memory retrieval in a spatial memory paradigm. Ten rats were used per group and the first group was trained and sacrificed five min, the second six hours following retrieval in the Morris Water Maze (MWM) and the third and fourth group were untrained, yoked controls. Hippocampi were taken and free AA were determined using a well-established HPLC protocol.

Beta-alanine and citrulline levels were higher in trained rat hippocampi, during both, early and late phase of memory retrieval. Taurine, methionine, cysteine, lysine and ornithine levels were higher in yoked rats at the late phase while tyrosine was higher in yoked rats during the early phase. There were no significant correlations between time spent in the target quadrant and any of the AA levels. Herein, an AA pattern, different between yoked and trained animals at early and late phase of memory retrieval is shown, indicating probable involvement of different AA pathways in animals trained and untrained in the MWM. The results may be useful for the interpretation of previous studies and the design of future experiments to identify amino acids as possible targets for modulating spatial memory.

1. Introduction

A series of amino acids (AA) that are not neurotransmitters themselves or direct precursors of neurotransmitters is associated with spatial memory formation. Therefore, these AA can be used as potential neuropharmaceuticals. Moreover, AA serve as important neuropharmacological tools and probes to study brain function. Interestingly, changes of several amino acids have been shown to be modified during memory formation per se: Tryptophan depletion was shown to interfere with memories but did not modify spatial memory [1] while long-term tryptophan administration improved cognitive performance [2]. Likewise, tyrosine depletion is known to attenuate dopamine function in healthy volunteers [3] and spatial memory is impaired [4]. It is well-documented and widely accepted

Abbreviations: MWM, Morris water maze; HPLC, high performance liquid chromatography; AA, amino acids.

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that arginine metabolism is involved in memory formation through nitric oxide signaling [5–9] and arginine metabolites L-citrulline and agmatine are changed during memory formation [10].

Several treatment and supplementation studies with amino acids have been carried out. For example, the arginine metabolite creatine was shown to improve cognitive performance in elderly individuals [11]. It was revealed that the hippocampal arginine metabolite, agmatine, was involved in spatial memory [12] and this metabolite also improved spatial working memory in the aging rat [13] and indeed, hippocampal agmatine levels increased during spatial learning [14,15]. In contrast, chronic administration of branched-chain amino acids was reported to impair spatial memory in the rat [16].

Although there is evidence for the involvement of several naturally occurring AA in neurological function, there is limited information about the role of beta-alanine in spatial memory. Therefore, the objective of this study was to determine hippocampal levels of beta-alanine in a spatial memory paradigm during an early and a late retrieval phase. Indeed, beta-alanine levels were increased in trained rats during both, the early and the late phase of memory retrieval in the MWM in the rat, thus proposing a probable role for this AA as a potential neurotransmitter in the brain and in turn indicating involvement of glycine receptors [17].

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats were used for the studies. Rats used were aged between 12 and 14 weeks and were obtained from and maintained in cages made of Makrolon and filled with autoclaved woodchips in the Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna. An autoclaved standard rodent diet (Altromin, Germany) and water in bottles was available ad libitum. The room was illuminated with artificial light at an intensity of about 200 lx in 2 m from 5 am to 7 pm. MWM test was performed between 8:00 h and 13:00 h. Forty animals were divided into four groups. Two groups were trained and the other two groups were used as untrained yoked controls that swam the same time as their trained counterparts.

All procedures were carried out according to the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Federal Ministry of Education, Science and Culture, Austria. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Morris water maze (MWM)

The MWM was performed as described by Patil [18] and Sase [19] with minor modifications. The MWM consisted of a circular pool (150 cm diameter, wall depth 60 cm) in which rats were trained to escape from water by swimming to a hidden platform (1.5 cm beneath water surface) whose location can only be identified using distal extra-maze cues attached to the room walls. Water temperature was maintained at 21 ± 1 °C.

The pool was divided into four quadrants by a computerized tracking/image analyzer system (video camcorder: 1/3″ SSAM HR EX VIEW HAD coupled to the computational tracking system: TiBeSplit). The platform was placed in the middle of the quadrant and remained at the same position during the training experiment.

The spatial acquisition phase consisted of four training trials per day and four training days. Rats were released with their heads facing the pool wall from the four compass locations (NE, NW, SW, and SE randomly), and allowed to swim and search for the platform for 120 s. If rats did not locate the platform after 120 s, animals were manually placed on the platform and allowed to remain on it for 30 s. Each animal was then returned to its cage for 20 min before its next trial. On the first training day, rats were given an acclimatization training session in the water maze; rats were placed on the hidden platform, were allowed to swim for 30 s, and were guided subsequently back to the platform, climbing onto the platform. The latency and path length and speed to reach the hidden platform was recorded.

On day 5, animals received a probe trial, in which the platform was removed. Rats were released from NE start point and allowed to swim freely for 60 s. The time spent in each quadrant was calculated.

Yoked controls were placed in the water maze to swim the same amount of time as their trained partners, but without a platform being present to climb onto. Animals were exposed to the same spatial cues, but without an escape platform, therefore rats did not develop an association between the extra-maze cues and the location of the platform. Yoked controls ruled out possible differences due to stress and physical effort (swimming) produced in the maze.

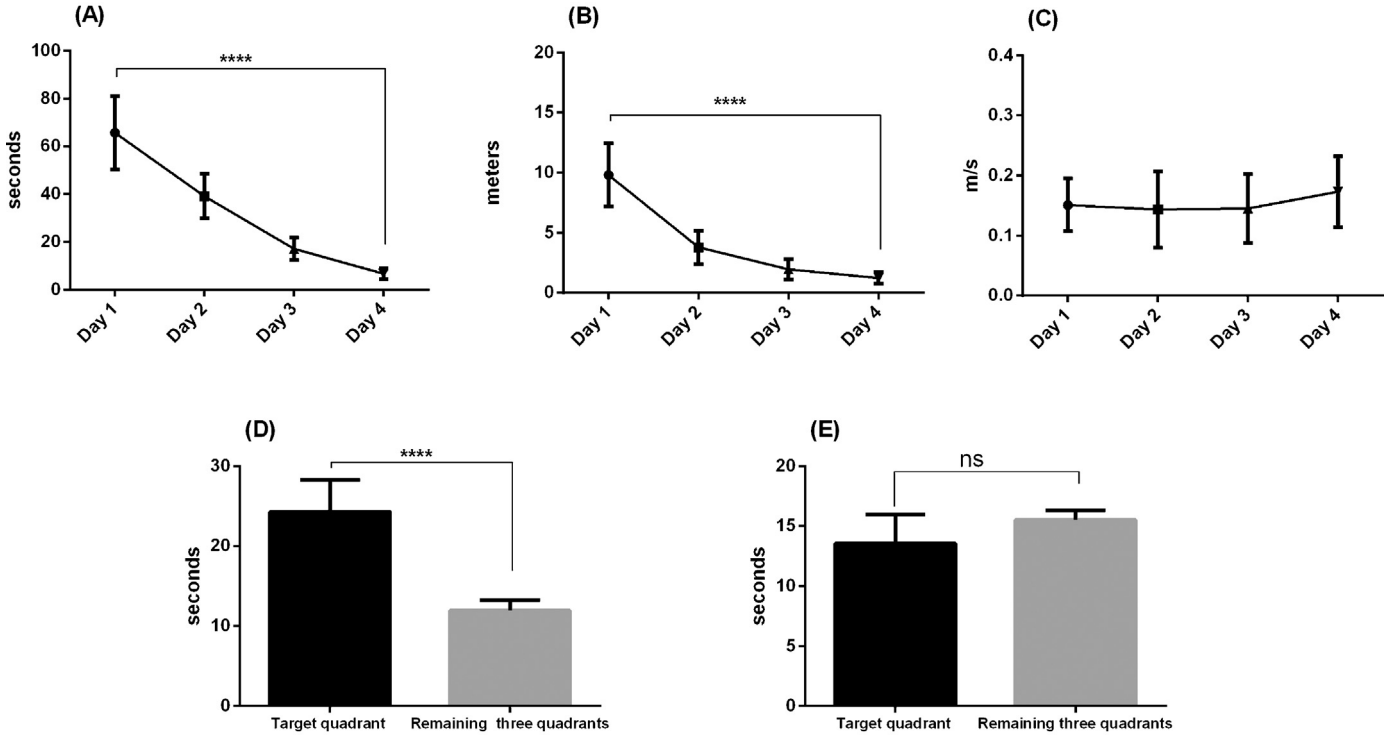

Fig. 1. Results of Morris water maze.
Learning phase in the MWM (Mean ± Standard deviation): (A) Latency (s) to reach the platform. (B) Path length (m) to reach the platform. (C) Average speed (m/s) during the learning phase, (D) Probe trial of trained and (E) Probe trial of yoked rats in the MWM.
*$P < 0.05$; **$P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns – not significant.