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Research report

Microinjection of histamine into the cerebellar vermis impairs emotional memory consolidation in mice

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ABSTRACT

The biogenic amine histamine is an important neurotransmitter in the central nervous system that has been implicated in learning and memory processes. Experimental evidence indicates that the role of the cerebellum may be more complex than the simple regulation of motor responses, and recent studies have demonstrated significant involvement of the cerebellum in emotional memory consolidation. This study investigated the effect of histamine microinjected into the cerebellar vermis on emotional memory consolidation in mice in the elevated plus-maze (EPM). The cerebellar vermis of male mice (Swiss Albino) were implanted with guide cannulae. The mice weighed between 25 and 30 g. After three days of recovery, behavioral tests in the EPM were performed on two consecutive days; the testing periods were called, Trial 1 and Trial 2. Immediately after Trial 1, the animals received microinjections of histamine in the cerebellar vermis (0.54, 1.36, 2.72, and 4.07 nmol/0.1 μl). On both days, the test sessions were recorded to enable analysis of behavioral measures. The decrease in open arm exploration (% entries and % time spent in the open arms) in Trial 2 relative to Trial 1 was used as a measure of learning and memory. The data were analyzed using One-way Analysis of Variance (ANOVA) and Duncan's tests. The percentage of open arm entries (%OAE) and the percentage of time spent in the open arms (%OAT) were reduced in Trial 2 relative to Trial 1 for the control group; the same was true for the group that was microinjected with histamine at doses of 0.54 (%OAE and %OAT) and 1.36 nmol (%OAT). However, when the animals received histamine at doses of 2.72 and 4.07 nmol, their open arm exploration did not decrease. No significant changes were observed in the number of enclosed arm entries (EAE), an EPM index of general exploratory activity. These results suggest that there is a dose-dependent inhibitory effect of histamine microinjected into the cerebellar vermis on emotional memory consolidation.

1. Introduction

Histamine is a biogenic amine and an important neurotransmitter–neuromodulator in the central nervous system (CNS) [13,22]. Recent evidence has clearly established that histamine and its receptors are involved in learning and memory. Numerous experiments using different learning models and species suggest that histamine may be important for several stages of memory formation and memory retrieval during different tasks, and they emphasize the role of histamine in the physiological mechanisms of memory [1,2,23,24,39]. However, these studies have used many different behavioral tasks and produced contradictory results; for instance, both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavior studies [1,6,15,36,46]. The mechanisms underlying these differences seem to be very complex, and the differences may be due in part to the methods used and the approaches selected in the experiments [19].

The central histaminergic nervous system originates from the tuberomammillary nucleus of the hypothalamus, and in many species, it widely innervates almost the whole brain including the cerebellum and other subcortical motor structures [38]. Previous studies have shown that the histamine-containing fibers project from the tuberomammillary nucleus to the cerebellar cortex and

Abbreviations: CNS, central nervous system; EPM, elevated plus-maze; OAE, open arm entries; OAT, open arm time; %OAE, percentage of open arm entries; %OAT, percentage of open arm time; EAE, enclosed arm entries; EAT, enclosed arm time; %EAT, percentage of enclosed arm time; CT, central area time; SAP, stretched-attend postures.

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the deep cerebellar nucleus and that the highest density of histaminergic terminations is in the vermis and flocculus [21,45]. A moderately dense network of histamine fibers has been seen in the molecular and granular layers of the cerebellum in several species including humans [14]. These fibers run parallel to the Purkinje cell layer after traversing it perpendicularly. Both Purkinje cells and neurons in the nucleus interpositus have H2 receptors [37], and granule cells are excited through both H1 and H2 receptor activation [43].

The cerebellum has traditionally been considered an important subcortical motor structure, but several lines of evidence support the view that the role of the cerebellum is more complex than previously thought and includes more than just the regulation of motor responses [34]. An increasing number of studies have demonstrated its involvement in cognitive and emotional function [35]. Functional neuroimaging studies and studies of patients with cerebellar lesions have been conducted to elucidate the role of the cerebellum in the processing of emotion [35,42,44]. According to Sacchetti et al. [32], the fact that there is a functional interconnection between the cerebellar vermis and the hypothalamus, amygdala, and hippocampus suggests that the cerebellum may play a role in an integrated network regulating emotional behavior. Moreover, Ruediger et al. [29] demonstrated that fear conditioning learning is specifically correlated with the growth of feedforward inhibition connectivity in hippocampal and cerebellar circuits.

Experimental evidence indicates that the cerebellum plays a role in emotional learning. The capacity to learn and retain fear-conditioned responses was investigated in *hotfoot* mutant mice. These animals are characterized by a primary deficiency in the synapses made by the parallel fibers onto the Purkinje cells. In these mutant mice, the cerebellar dysfunction impairs learning, which suggests that these synapses are involved in fear memory consolidation [31]. Studies have related the cerebellar vermis to emotional memory consolidation. In one study, vermis inactivation caused amnesic effects after a fear conditioning task [30]. Thus, the participation of the vermis in emotional memory is independent of its role in sensory or motor processes, and the vermis may represent an interface between sensory stimuli, emotional state, and motor responses [30,34].

Studies have demonstrated the relationship between the histaminergic system and the cerebellum. Shen et al. [37] demonstrated that histamine excites the cerebellar interpositus nucleus cells via the histamine H2 receptor mechanism. They suggested that the hypothalamocerebellar histaminergic fibers may modulate neuronal activity in the cerebellum. The results of a study by Tian et al. [43], revealed that histamine excites cerebellar Purkinje cells via H2 receptors and that the histaminergic fibers may play an important role in functional aspects of the cerebellum. In spite of these investigations, there have been no reports on the cerebellar histaminergic system and learning and memory processes.

The elevated plus-maze (EPM) is an animal model test of anxiety based on rodents' natural aversion to open spaces [18]. A general aspect of EPM exploration is that animals enter and spend less time exploring the open arms [7]. The inclusion of a retest session has been made in recent years, which is consistent with the assumption that there is a learned component underlying the exploratory behavior during EPM re-exposure [8]. According to File et al. [8], after the initial exploration of the apparatus, rodents acquire, consolidate and retrieve some kind of memory related to exploration of potentially dangerous areas of the maze. Bertoglio and Carobrez [4] use Trial 1/2 protocol to show that after a single prior non-drugged experience in the maze, mice exhibit significantly reduced open arm activity in a second trial. An increase in open arm avoidance with repeated maze exposure has been observed in several studies [7,10,16] and has been used as a measure of learning and memory [12,16,36].

The present study was designed in view of these findings to investigate the action of histamine microinjected into the cerebellar vermis on emotional memory consolidation in mice using Trial 1/2 protocol in the EPM.

2. Material and methods

2.1. Animals

Male Swiss mice (Federal University of São Carlos, UFSCar, SP, Brazil) weighing 25–35 g at the beginning of the experiments were housed in polypropylene cages ($31 \times 20 \times 13$ cm) in groups of five and maintained under a 12 h light cycle (lights on at 7:00 a.m.), in a controlled environment at temperature 23 ± 1 °C and humidity 50 ± 5%. Food and drinking water were provided *ad libitum*, except during the brief test periods. All mice were experimentally naive, and the experimental sessions were conducted during the light period of the cycle (9:00–13:00 h).

2.2. Drugs

Histamine dihydrochloride (Sigma Chemical Co., USA) was prepared in a vehicle of physiological saline. Saline solution was used as an experimental control. The doses of histamine were based on previous research [28] and on pilot work in our own laboratory. The substances were coded, and the codes were unknown to the experimenter during the tests and behavioral analysis.

2.3. Surgery and microinjection

Each mouse was implanted with a single 7 mm stainless steel guide cannula (25 gauge) under ketamine chloridrate and xylazine solution anesthesia (100 mg/kg and 10 mg/kg, respectively, delivered via i.p. injection). The stereotaxic coordinates for the cerebellar vermis were 6.5 mm posterior to bregma, 0 mm lateral to the midline, and 2.0 mm ventral to skull surface [9]. The guide cannula was fixed to the skull using dental acrylic and Jeweler's screws. A dummy cannula (33 gauge stainless steel wire) was inserted into the guide cannula at the time of surgery and served to reduce the incidence of occlusion. Postoperative analgesia was provided for 3 days by adding acetaminophen (200 mg/ml) to the drinking water in a ratio of 0.2 ml acetaminophen to 250 ml water (i.e., the final concentration was 0.16 mg/ml). Saline and drug solutions were infused into the cerebellar vermis using a microinjection unit (33 gauge cannula; Cooper's Needleworks, Birmingham, UK), which extended 2.0 mm beyond the tip of the guide cannula. The microinjection unit was attached to a 5 μl Hamilton microsyringe via polyethylene tubing (PE-10), and the administration was controlled by an infusion pump (Insight Equipamentos Científicos Ltda, Brazil) programmed to deliver a volume of 0.1 μl over a period of 60 s. The microinjection procedure consisted of gently restraining the animal, inserting the injection unit, infusing the solution, and keeping the injection needle *in situ* for a further 60 s to avoid reflux. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble inside the PE-10 tubing.

2.4. Apparatus

The EPM used was similar to that originally described by Lister et al. [18]. The EPM consisted of two open arms ($30 \times 5 \times 0.25$ cm) and two enclosed arms ($30 \times 5 \times 15$ cm) connected to a common central platform (5×5 cm). The apparatus was made of crystal acrylic and was raised to a height of 38.5 cm above floor level. All tests were conducted under moderate illumination (77 lx) as measured on the central platform of the EPM and in an environment isolated from the rest of the room by a black protective curtain.

2.5. Experimental procedure

Three days after surgery, the animals were transported to the experimental room and left undisturbed for at least 1 h before testing to facilitate adaptation. The test was performed on two consecutive days, and the trials in the EPM were denoted: Trial 1 and Trial 2. Mice were individually placed on the central platform of the maze facing the open arm and were able to explore the maze for 5 min. In Trial 1, immediately after the exposure to the EPM, animals received microinjection of saline or histamine in the cerebellar vermis (0.54, 1.36, 2.72 and 4.07 nmol/0.1 μl). Twenty-four hours later (Trial 2), mice were re-exposed to the EPM under the same experimental conditions, but they did not receive any injection. Between subjects, the maze was thoroughly cleaned with 5% ethanol and a dry cloth.

2.6. Behavioral analysis

All sessions were video recorded by a digital camera linked to a computer in an adjacent room. Images were analyzed by a highly trained observer using X-PLO-RAT, an ethological analysis pack developed at the Laboratory of Exploratory Behavior USP/Ribeirao Preto [11]. Behavioral parameters were defined in a way that was consistent with previous studies [18,26] and included the following: the frequency of open- and enclosed-arm entries (OAE and EAE) (an entry was defined as the entry of all four of an animal's paws into an arm) and total time