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Posttraining systemic administration of the histone deacetylase inhibitor sodium butyrate ameliorates aging-related memory decline in rats

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Abstract

Here we show that a systemic injection of the histone deacetylase inhibitor (HDACi) sodium butyrate (NaB) ameliorated an aging-associated deficit in object recognition memory in rats when the injection was given immediately, but not 6 h after training. NaB had no effect in younger rats with normal memory retention. The results indicate that HDACis can ameliorate aging-related memory impairments by influencing the early consolidation phase of memory formation.

Keywords

Sodium butyrate; Histone deacetylase; Recognition memory; Epigenetics; Memory consolidation

Memory formation involves changes in neuronal gene expression. Accumulating evidence indicates that chromatin modification is a key epigenetic mechanism regulating gene transcription during the stabilization of long-term memories. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play opposing roles in modifying chromatin. HATs increase the access to genomic DNA by relaxing chromatin, whereas HDACs induce chromatin condensation and repress gene transcription. HDAC inhibitors (HDACis), initially developed as potential anticancer agents [14], have been shown to enhance memory,

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or ameliorate deficits in experimental models of memory dysfunction, by inducing a histone hyperacetylated state (for recent reviews, see [2, 12, 21]).

Memory decline related to aging has been recently associated with changes in epigenetic modification of chromatin [16 – 18, 23], and administration of a HDACi restored memory-associated regulation of gene expression and improved memory in aged mice [16]. Sodium butyrate (NaB) is an HDACi that enhances formation and extinction of memory when given systemically before training in rats and mice [10, 11], and improves memory deficits in models of neurodegenerative disorders and acute brain injury [1, 3, 9]. However, previous studies have not examined whether systemic HDAC inhibition can ameliorate aging-related cognitive decline by influencing memory consolidation.

In the present study, we examined the effects of posttraining systemic administration of NaB on the formation of novel object recognition (NOR) memory in young adult and aged rats. We found that aged rats showed a deficit in 24-h retention of NOR, which was rescued by NaB given immediately after training, but not at a later posttraining interval. The results suggest that HDACis can rescue aging-associated cognitive decline by influencing the early phase of memory consolidation.

Young adult (age 3 months) or aged (age 24 months for the experiment with immediate posttraining injections; age 18 months for the experiment with 6-h delayed injections) male Wistar rats were obtained from our institutional certified breeding colony (CREAL-UFRGS). Animals were kept in groups of 3 per cage in a plastic cage with sawdust bedding, with room temperature of 22 ± 1 °C and a 12h light/dark cycle, with standardized pellet food and water *ad libitum*. All behavioral experiments took place between 9 AM and 5 PM. All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals NIH Guide for Care and Use of Laboratory Animals (NIH publication number 80–23 revised 1996) and approved by the institutional animal care committee.

NOR training and testing took place in a 40 cm × 50 cm open field surrounded by 50 cm high walls made of plywood with a frontal glass wall. The floor was covered with sawdust. Objects used in training and testing trials presented distinctive colors and shapes and consisted of pairs of identical cans, small glass bottles, or blocks made with plastic Duplo Lego toys. The different objects and their positions were counterbalanced across experiments and behavioral trials, and all objects had a height of about 10 cm. Between trials the objects were washed with a 70% ethanol solution. Exploration was defined as sniffing or touching the object with the nose and/or forepaws, sitting on the object was not considered exploration. Training and test procedures followed the general methods described in previous reports [4, 5, 19]. Briefly, rats were left to explore the empty arena for 2 minutes in the first day (habituation). Twenty-four hours after habituation, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. Animals were left to explore the objects until they had accumulated 30 s of total object exploration time a maximum of 20 min. On a memory retention test trial given 24 h after training, rats explored the open field for 5 minutes in the presence of one familiar (A) and one novel (B) object. Object exploration was measured by one experimenter blind to group treatment assignments. A recognition index calculated for each animal was expressed by the ratio $TB/(TA + TB) * 100$ [TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B]. Because all rats were trained to a criterion of 30 s of total time exploring both objects, there were no differences among experimental groups in the amount of object exploration. This protocol was used to avoid the possible interference on learning of age-related alterations in locomotion or motivation in learning [4, 5, 19].

Rats received a single intraperitoneal (i.p.) injection of saline (NaCl 0.9%) or NaB (1.2 g/kg) dissolved in saline in a 2.0 ml/kg injection volume immediately after training. In another experiment (delayed injection), aged rats received the injection of saline or NaB 6 h after training. The dose of NaB was chosen on the basis of previous studies [10, 11, 13, 22] as well as pilot experiments performed in our laboratory. NaB 98% was purchased from Sigma, St. Louis, MO, USA.

Data are expressed as mean $\pm$ S.E.M. exploratory preferences. Comparisons between groups were performed using a Kruskal-Wallis analysis of variance followed by Mann-Whitney *U*-tests, two-tailed when necessary. Comparisons between trials (preference for the novel object in the test trial compared with exploratory preference for object A2 in the training trial) within the same group were performed by Wilcoxon tests [4, 5, 19]. Comparisons between rats treated with NaB and their respective controls in the time required to reach criterion during training were made with Student *t*-tests for independent samples. In all comparisons, $P < 0.05$ was considered to indicate statistical significance.

Results for young adult rats are shown in Fig. 1. There was no significant difference between rats given saline and NaB in the time required to reach criterion (mean $\pm$ S.E.M. time (s) was 176.8 ± 11.9 in the saline group and 186.0 ± 6.4 in the NaB group; $P = 0.51$). Both experimental groups showed significant memory retention as revealed by the comparison between exploratory preferences between the training and test trials (saline, $P = 0.005$, $n = 10$; NaB, $P = 0.008$, $n = 9$). There were no significant differences between groups (training, $P = 0.45$; test, $P = 1.00$). Results for aged rats given an immediate posttraining injection are shown in Fig. 2A. There was no significant difference between groups in the time required to reach criterion (mean $\pm$ S.E.M. time (s) was 573.0 ± 118.8 in the saline group and 596.4 ± 120.5 in the NaB group; $P = 0.89$). Control aged rats given saline immediately after training showed impaired 24-h retention of NOR memory: there was no significant difference between performance in training and test trials, although it felt short of significance ($P = 0.074$, $n = 10$). In contrast, rats given NaB showed significant memory retention in the test trial ($P = 0.008$, $n = 9$). There was a significant difference in exploratory preference between groups in the test trial ($P = 0.035$), but not in the training trial ($P = 0.50$). The mean percentage of time exploring the novel object in the retention test for aged rats given NaB (about 75%) was comparable to those of young adult rats given either saline or NaB (about 70% in both groups). The results indicate that aging is associated with an impairment of 24-h retention of NOR, and this impairment is rescued by a single systemic injection of NaB given immediately, but not 6 h, after training. In contrast, in younger animals that showed normal levels of memory retention, NaB did not produce further enhancement of NOR retention.

To verify whether the beneficial effects of NaB were specific for the early phase of consolidation, an additional experiment was carried out in which injections were given at a delayed time point (6 h) after training. Results are shown in Fig. 2B. There was no significant difference between groups in the time required to reach criterion (mean $\pm$ S.E.M. time (s) was 548.5 ± 138.5 in the saline group and 667.0 ± 84.7 in the NaB group; $P = 0.49$). Neither the saline nor the NaB group showed significant memory retention assessed by training-test-differences (saline, $P = 0.92$, $n = 6$; NaB, $P = 0.74$, $n = 7$). In addition, there were no significant differences between groups (both P s = 0.73 for training and test trials). These results confirm that aged rats show impaired NOR memory and indicate that NaB does not improve the impairment when given at a later time point after training.

Because all animals were trained to a criterion of object exploration, the differences between young and aged rats given saline observed in the present study were unlikely to be related to differences in acquisition, locomotion, or motivation. In addition, there were no differences

in the time of context exposure required to reach criterion between rats treated with NaB and their respective controls. Direct comparisons between young and aged rats were not performed because the experiments using young and adult rats were carried out separately. However, aged rats clearly took a longer exposure time to reach the 30-s criterion of object exploration. In spite of the similar amounts of object exploration during training in all groups, we cannot rule out the possibility that the longer context exposure time in aged rats has influenced the differential effects of NaB in young and aged rats.

Since NaB injections were given after training, the NaB-induced improvement of performance observed in aged rats cannot be explained by drug-induced alterations in acquisition, anxiety, locomotion, motivation, or other sensorial or motor parameters. Also, the lack of effect of NaB when given 6 h after training in aged rats indicates that the improving effect of immediate posttraining NaB on 24-h retention was not produced by long-lasting effects of NaB that could influence performance by affecting aspects of brain function and behavior unrelated to memory. Finally, systemic administration of NaB to rodents given behavioral training has been used in several previous studies without any evidence indicating that NaB can produce drug-induced conditioned aversion that could influence performance.

Several studies have shown that systemic or intracerebral administration of HDACis can produce memory-enhancing effects in normal animals as well as in models of brain disorders [1, 3, 7, 9–11, 22, 24], and it was recently shown that HDACis can ameliorate aging-related memory decline in mice [16]. Many of the studies investigating the effects of HDACis on memory have used systemic administration of NaB to produce memory enhancement via HDAC inhibition. Although a few previous studies have indicated that NaB can enhance memory when given after training [6, 20, 22], there is no previous evidence that HDACis can modify aging-related memory deficits by specifically influencing consolidation. The present results add to the previous evidence by showing that, in rats, acute systemic administration of NaB can rescue aging-related impairment in object recognition memory when given after training.

A previous study in *Chasmagnathus granulatus* crabs has found that NaB enhanced memory consolidation at two time windows after training, immediately and 6 h after training [6]. In contrast, we found no effect of NaB injected 6 h after training in 18-month old rats with impaired retention of NOR memory. This difference might be related to species-specific differences in the action of NaB and/or in the influence of histone acetylation in memory consolidation. Additional studies are required to further characterize the time window for the effects of HDACis in consolidation in rodents. Another difference between the present study and previous findings is that, in our experiment, NaB failed to affect NOR memory measured 24 h after training in young adult rats. Stefanko et al. (2009) showed a significant enhancement of 24-h NOR retention after posttraining injection of 1.2 g/kg NaB in young adult mice. This discrepancy might be explained by the lower retention index displayed by control mice in the study by Stefanko et al. (2009), which used a sub-threshold condition for training (i.e., a 3-min training trial), whereas in the present study young saline-treated rats were given a stronger training protocol and showed significant retention. Under conditions in which control animals show high retention scores for NOR memory, there might be a “ceiling effect” that can prevent cognitive enhancers from further improving memory.

Several molecular mechanisms modified in response to chromatin relaxation might be involved in the effects of HDACis on memory in aged rats. For example, evidence indicates that the memory-enhancing effects of HDACis is mediated primarily by activation of genes regulated by the CREB:CREB-binding protein (CBP) transcriptional complex [8, 15, 24]. Memory impairment associated with aging in mice was specifically related to changes in

histone H4 lysine 12 (H4K12) acetylation, leading to altered expression of several genes involved in memory consolidation. Importantly, HDACis (including NaB) that restored normal H4K12 also rescued aging-related memory impairment [16]. Recent evidence suggests that aging is also associated with aberrant changes in methylation of the *Arc* gene, leading to decreased transcription of *Arc*, an immediate early gene required for memory consolidation [18]. One can speculate that restoration of *Arc* expression might be a mechanism involved in HDACi-induced amelioration of memory dysfunction associated with aging. Advances in the detailed understanding of alterations in specific histone subtypes in aging and pathological conditions, and investigation of the actions of HDACis on these alterations, will allow the identification of the most promising HDACis for ameliorating cognitive dysfunction in a clinical setting.

In conclusion, the present results indicate that HDACis can ameliorate aging-related memory impairments by influencing the early consolidation phase of memory formation.

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Fig. 1.

Posttraining administration of the histone deacetylase inhibitor (HDACi) sodium butyrate (NaB) does not affect retention of novel object recognition (NOR) in young adult rats. Male Wistar rats (age 3 months) were trained in a NOR task and tested for retention 24 h later. Immediately after training, animals received a single intraperitoneal (i.p.) injection of saline (NaCl 0.9%, $n = 9$) or NaB (1.2 g/kg, $n = 9$) dissolved in saline. ## $P < 0.01$, comparison between training and test within the same group. There was no significant difference between groups.

Fig. 2.

Posttraining administration of the histone deacetylase inhibitor (HDACi) sodium butyrate (NaB) rescues aging-associated memory impairment in rats. Aged male Wistar rats (age 18 or 24 months) were trained in novel object recognition (NOR) task and tested for retention 24 h later. Either immediately after (A) or 6 h after (B) training, animals received a single intraperitoneal (i.p.) injection of saline (NaCl 0.9%, $n_s = 10$ for immediate posttraining injections and 6 for 6 h delayed injections) or NaB (1.2 g/kg, $n_s = 9$ for immediate posttraining injections and 7 for 6 h delayed injections) dissolved in saline. ## $P < 0.01$, comparison between training and test within the same group. * $P < 0.05$, comparison between experimental groups within the same trial.