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Histone Deacetylase 2 in the Mouse Hippocampus: Attenuation of Age-Related Increase by Caloric Restriction

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Abstract

The aging process in the hippocampus is associated with aberrant epigenetic marks, such as DNA methylation and histone tail alterations. Recent evidence suggests that caloric restriction (CR) can potentially delay the aging process, while upregulation of antioxidants may also have a beneficial effect in this respect. We have recently observed that CR attenuates age-related changes in the levels of the epigenetic molecules DNA methyltransferase 3a, 5-methylcytidine (5-mC) and 5-hydroxymethylcytosine in the mouse hippocampus while overexpression of the antioxidant Cu/Zn superoxide dismutase 1 (SOD1) does not. However, the impact of aging on the levels of histone-modifying enzymes such as histone deacetylase 2 (HDAC2) in the hippocampus has not been studied in much detail. Here, we investigated immunoreactivity (IR) of HDAC2 in three subregions of the hippocampus (dentate gyrus, CA3 and CA1-2) of mice taken from large cohorts of aging wild-type and transgenic mice overexpressing normal human SOD1, which were kept under normal diet or CR from weaning onwards. Independent from the genotype, aging (between 12 and 24 months) increased levels of HDAC2 IR in the hippocampus. Moreover, CR prevented this age-related increase, particularly in the CA3 and CA1-2 subregions, while SOD1 overexpression did not. Quantitative image analyses showed that HDAC2 IR correlated positively with 5-mC IR while these markers were shown to colocalize in the nucleus of hippocampal cells. Together with recent literature reports, these findings suggest that altered levels of epigenetic regulatory proteins including HDAC2 regulate age-related changes in the mouse hippocampus and that CR may prevent these age-related changes.

Keywords

Aging; epigenesis; histone deacetylase 2 (HDAC2); caloric restriction; hippocampus

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

INTRODUCTION

Several molecular and morphological alterations have been observed in the aging brain and have been associated with cognitive impairment and an increased risk for neurodegenerative disorders [1–4]. Among others, age-associated changes in gene expression involve downregulation of synaptic genes and genes encoding neurotrophins as well as upregulation of genes mediating the immune response [5–7]. Dysfunctional epigenetic regulation of gene expression, especially through DNA (hydroxy)methylation and histone modifications, have recently been implicated with such changes [8]. For example, we have recently reported age-related increases in hippocampal immunoreactivity (IR) of DNA methyltransferase 3a [9], the DNA methylation marker 5-methylcytidine (5-mC) [10] and the DNA hydroxymethylation marker 5-hydroxymethylcytosine (5-hmC) [11].

While caloric restriction (CR) has been shown to increase life span and delay the onset of age-related neurodegeneration in various species [12–16], upregulation of endogenous antioxidant activity is believed to act beneficially by reducing oxidative damage [17–20]. We tested the effects of CR and overexpression of the normal human antioxidant Cu/Zn superoxide dismutase 1 (SOD1) in large cohorts of aging mice (the same mice used in the present study) on the observed age-related changes in hippocampal levels of Dnmt3a, 5mC and 5-hmC and found that they all could be prevented by CR, but not by SOD1 overexpression.

Next to DNA methylation and hydroxymethylation changes, histone tail modifications encompass a wide range of variation in epigenetic regulation. In particular, histone acetylation, mediated by histone acetyltransferases (HATs), and histone deacetylation, mediated by histone deacetylases (HDACs), represent key processes in the regulation of gene expression [8, 21]. Several HDACs, particularly HDAC2, have been implicated in physiological and pathological aging of the brain [22, 23]. HDAC2 belongs to the class I of histone deacetylases that are responsible for the removal of acetyl groups from histone tails, generally promoting transcriptional repression [21]. HDAC2 was recently identified as a negative regulator of memory and synaptic plasticity while age-related memory impairment has been linked with histone acetylation deregulation [22–26]. Moreover, HDAC2 was shown to decrease acetylation and block the expression of genes related to learning and memory, while HDAC2 knockdown reversed synaptic deficits and associated cognitive impairment in the Ck-p25 mouse model of neurodegeneration [27]. In support of this notion, various other studies in rodents have revealed the potentially protective effects of HDAC inhibitors (HDACi) against age-related cognitive impairment and various types of neurodegenerative disorders [25, 28–31]. To date, the exact role of HDAC2 in brain aging is far from being completely understood. Furthermore, it is unknown whether altered levels and/or function of HDAC2 could mediate the beneficial effects of protective strategies such as CR.

Using the same experimental design as in our previous work [9–11] the present study investigated hippocampal IR of HDAC2 in the dentate gyrus (DG), CA3, and CA1-2 of the mouse hippocampus. The aim of the present study was to examine i) the regional and cellular localization of HDAC2 in the hippocampus, ii) the effects of aging, CR and overexpression of SOD1 on the levels of HDAC2 in the hippocampus, and iii) to investigate whether altered levels of HDAC2 IR in the hippocampus correlate with altered levels of 5-mC IR, another marker of DNA methylation that we have investigated in a separate study [10].

MATERIALS AND METHODS

Animals

A total of 48 male mice were used for the present study. These mice were derived from a cohort of 240 aging male mice that consisted of four groups based on genotype and diet: 1) WT (C57Bl6J) mice on CD (WT-CD), 2) SOD1 mice on CD (SOD-CD), 3) WT mice on CR (WT-CR) and SOD1 mice on CR (SOD-CR). Per group, 6 mice were euthanized at 12 and 24 months of age, thus yielding a total of 8 groups of 6 mice each for histological analyses.

Detailed descriptions of the transgenic mice generation, preparation and composition of the diets and weight and survival curves have been reported previously [9, 11, 32]. Briefly, the transgenic mice were generated on a C57Bl6J background and were carrying 7 copies of the entire human SOD1 sequence, inserted into chromosome 3, resulting in an increased enzyme activity in the brain and other tissues [33]. In order to fully monitor the caloric intake, the CD covered approximately 85% of the *ad libitum* consumption, while the calorie restricted diet entailed a 50% reduction in calories. The animals were housed individually, with *ad libitum* access to water, on 12/12 hours light/ dark cycle kept under standard temperature, humidity, and specified pathogen free (SPF) conditions. All experiments were approved by the Animals Ethics Board of Maastricht University.

Tissue Processing

Procedures regarding brain processing have been described in detail previously [9, 32]. In brief, mice were transcardially perfused with tyrode solution and two fixative solutions (4% paraformaldehyde, 0.9% NaCl, 1% acetic acid and 8% paraformaldehyde, 0.9% NaCl, 1% acetic acid). Brains were removed and postfixed for 24 hours in 8% paraformaldehyde (at 4° C), hemisected along the midsagittal line, cryoprotected in sucrose solutions (10, 20, and 30% sucrose in Tris-HCl buffer, 2×12 hours per solution at 4° C) and embedded in Tissue Tek® (Sakura Finetek Europe, Zoeterwoude, The Netherlands). For the present study only the left brain halves were cut serially in 30 µm-thick free-floating coronal sections on a cryostat (type HM 500 OMV, Microm, Walldorf, Germany). For this study, parallel 1:10 series of sections were used and stored for further histological processing [9, 11, 32].

Immunohistochemical Detection of HDAC2

Using previously described protocols one series of sections was stained [10]. A rabbit monoclonal anti-HDAC2 (dilution 1:5000, ab32117, Abcam, Cambridge, UK) was used as a primary and a biotinylated donkey anti-rabbit (dilution 1:200, Jackson Westgrove, PA, USA) as a secondary antibody. The specificity of the commercially available anti-HDAC2 primary antibody has been confirmed previously using short interfering RNA (siRNA) inhibition [34]. In particular this antibody recognizes residues in the C-terminal of the HDAC2 protein.

Double immunofluorescence of HDAC2 and 5-mC was performed using the rabbit monoclonal anti-HDAC2 (dilution 1:5000, Abcam, Cambridge, UK) and a mouse monoclonal anti-5-mC (dilution 1:500; GenWay Biotech, San Diego, CA, USA) as primary antibodies. A donkey anti-rabbit Alexa 488 (dilution 1:200, Invitrogen, Eugene, Oregon, USA) and a donkey anti-mouse biotin (dilution 1:200, Jackson Westgrove, PA, USA) coupled with streptavidin Alexa 594 (dilution 1:400, Invitrogen, Eugene, Oregon, USA) were used for detection. In addition, the sections were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma Aldrich, Zwijndrecht, The Netherlands).

Analysis of HDAC2 IR

Mean intensity (inversed gray values) and surface area of HDAC2 IR were analyzed as described previously [9–11]. As indicated in (Fig. 1), two images from the CA1-2 region, two images from the CA3 pyramidal layer, and four images from the granule cell layer of the DG were taken at four different bregma levels (−1.58, −1.82, −3.40, −3.52), according to a mouse brain atlas [35], using a 40× objective. Thus, a total of 32 images were taken for every animal, with a digital camera (F-view; Olympus, Tokyo, Japan) connected to an Olympus AX70 brightfield microscope (analySIS; Imaging System, Münster, Germany). The ImageJ software program (version 1.42q, Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA), was used for the calculation of the mean intensities and surface areas. The mean intensities were calculated in arbitrary units and therefore the differences between groups in these units do not reflect the differences in actual levels of HDAC2 protein. Surface area measurements were corrected for previously reported volume reductions by age and CR [9, 32]. Surface area measurements were corrected by means of multiplying the volume of each subregion of each animal with the corresponding surface area measurement. Intensity measurements are independent of volume or cell number differences and therefore no adjustments were applied.

Imaging of HDAC2, 5-mC, and DAPI

Imaging of the triple labeling of HDAC2, 5-mC, and DAPI (Fig. 2) was achieved by collecting 16 µm-thick image stacks consisting of 80 confocal images (0.2 µm apart), made with a 60× objective (Olympus UPlanSApo; numerical aperture [NA] = 1.40, Olympus, Tokyo, Japan) and the SI-SD system, which consists of a modified Olympus BX51 fluorescence microscope with customized spinning disk unit (DSU Olympus), computer controlled excitation and emission filterwheel, three-axis accuracy computer-controlled stepping motor specimen stage (4×4 Grid Encoded stage, Ludl Electronic Products, Hawthorne, NY, USA), linear z-axis position encoder (Ludl), ultra-high sensitivity monochrome electron multiplier CCD camera (1000 × 1000 pixels, C9100-02, Hamamatsu Photonics, Hamamatsu City, Japan) and controlling software (Stereo Investigator; MBF Bioscience Williston, VT, USA). Projections and minor corrections in intensity and contrast were made with the Imaris software program (Bitplane AG, Zurich, Switzerland) [9]. Fig. (1) represents a “Virtual Slice” of the hippocampus (montage by collecting a series of contiguous images aligned, stitched and blended together) using a 20× objective (Olympus BX50 with UPlanApo objectives), a motor specimen stage (Ludl) and the Stereo Investigator software (MBF Bioscience).

Statistical Analysis

All data are presented as mean and standard error of the mean (SEM). The general linear model univariate analysis of variance was used for comparisons between groups, controlling for the main and interactive effects of age, genotype and diet (three-way ANOVA). Statistical significance was set at $\alpha = 0.05$. Pair-wise comparisons were performed with a Bonferroni post-hoc test. Correlation analyses between HDAC2 and 5-mC intensity measurements, that have previously been reported [10], were carried out by calculating the Pearson's correlation coefficient (r_p). The Statistical Package for the Social Sciences, (SPSS 17, SPSS Inc., Chicago, IL, USA) was used for all the statistical calculations. Graphs were built in GraphPad Prism (Version 4, GraphPad Software, San Diego, USA).

RESULTS

Qualitative Analysis of HDAC2 IR

HDAC2 IR was observed in all hippocampal subregions and particularly located in the nuclei of neurons and glial cells. Immunofluorescence labeling of HDAC2 and 5-mC showed that the two markers are colocalized (see yellow in Fig. 2D, 2H). Moreover, in accordance with previous observations, colocalization of 5-mC and DAPI was also observed, while there was no evident colocalization between HDAC2 and DAPI-dense heterochromatic DNA regions (Fig. 2).

Qualitative analysis, by inspection, of HDAC2 IR suggested an increase of HDAC2 IR from 12- to 24-month-old in CD mice in the DG, CA3 and CA1-2 hippocampal subregions, which was not observed in the CR mice (Fig. 3). Image analyses were performed to quantify these observations.

Quantitative Analysis of HDAC2 IR

HDAC2 IR Intensity—General linear model univariate analysis of variance showed an increase of HDAC2 IR intensity with aging from 12- to 24-month-old mice in all investigated regions ($p = 0.001$ for DG, $p = 0.008$ for CA3, and $p = 0.001$ for CA1-2; Fig. 4A–C). Furthermore, significant age $\times$ diet interactions were observed in the CA3 ($p = 0.026$) and CA1-2 ($p = 0.045$) regions (Fig. 4B–C). While no significant interaction between age and CR was found for HDAC2 IR in DG, it is interesting to note i) that 24-month-old mice fed with CR showed a similar mean value of HDAC2 IR in DG compared to 12-month-old mice fed with CR while the mean HDAC2 IR in 24-month-old mice fed with CD was higher than the mean HDAC2 IR in 12-month-old mice fed with CD, and ii) that the standard error of mean for HDAC2 IR in DG was relatively large compared to the data for CA3 and CA1-2. No significant effect of the SOD1 genotype was observed in any of the cases. In addition, post-hoc Bonferroni tests showed a significant increase of HDAC2 intensity from 12-to 24-month old WT mice (+11.2 %, $p = 0.049$).

Surface Area of HDAC2 IR—General linear model univariate analysis of variance of HDAC2 IR surface area revealed a significant main effect of diet ($p = 0.001$) and an age $\times$ diet interaction ($p = 0.046$) in the CA3 region only (Fig. 4E). Again, no significant effect of the SOD1 genotype was observed in any of the cases. In addition, post-hoc Bonferroni tests showed a significant decrease of HDAC2 surface area from 24-month-old SOD CD mice to 24-month-old SOD CR mice (−38.8 %, $p = 0.017$).

HDAC2 IR and 5-mC IR Correlation Analysis—Correlation analyses between intensities in 5-mC IR and HDAC2 IR showed a positive linear correlation in the CA3: ($r_p = 0.398$, $p = 0.006$) and the CA1-2 ($r_p = 0.384$, $p = 0.008$) regions (Fig. 5B–C), but not in the DG ($r_p = 0.230$, $p = 0.120$; Fig. 5A)

DISCUSSION

Qualitative analysis of HDAC2 IR showed nuclear HDAC2 IR in all hippocampal subregions, and suggested an age-related increase. Quantitative analyses indicated that HDAC2 IR indeed increased with aging, in particular in mice fed with CD, but not in mice under CR. No effects of the SOD1 genotype were found. HDAC2 IR correlated positively with 5-mC IR in the CA3 and CA1-2 subregions, and nuclear colocalization of these two markers was observed. However, HDAC2 IR was also observed in nuclear compartments devoid of 5-mC IR. The present study is the first to describe age-related changes in HDAC2

levels in the mouse hippocampus, and to describe the effects of CR and SOD1 overexpression.

Age-Related Increase in HDAC2 IR

The quantitative analyses showed a significant increase in HDAC2 IR with aging from 12 to 24 months in all investigated hippocampal subregions of mice fed with CD. The mechanism behind the increased levels of HDAC2 with aging is not yet clear, but it has been reported that HDAC2 is recruited at sites of increased DNA damage as part of the DNA damage response mechanisms [36]. Because increased DNA damage is a hallmark of aging [2, 37], it is possible that increased levels of HDAC2 are a secondary response to other age-related changes such as DNA damage. Alternatively, increased levels of HDAC2 may be associated with age-related dysregulation of histone acetylation, which has been demonstrated to contribute to age-related memory impairments [22, 25]. Peleg *et al.* reported that age-related memory impairment of 16-month-old mice is linked with impaired hippocampal H4K12 acetylation and associated expression of learning-related genes [25]. Overexpression of HDAC2 in mice (specifically in neurons) was shown to result in decreased spine density, synaptic number, and synaptic plasticity as well as impaired memory formation, while knocking out HDAC2 resulted in opposite effects [26]. Furthermore, increased cortical and hippocampal acetylation and methylation of histones H3 and H4 has been linked with improved memory after environmental enrichment in the CK-p25 transgenic mouse model of neurodegeneration [24]. This mouse model expresses the p25 activating subunit of cyclin-dependent kinase 5 in the basal forebrain and exhibits hyperphosphorylation of tau and neuronal loss [24]. Furthermore, increased HDAC2 levels have been observed in the CA1 subregion of this model, which were associated with down-regulation of expression of genes associated with memory and synaptic plasticity, such as *Arc*, *Bdnf* and *GluR1* [27]. Meanwhile, short-hairpin-RNA-mediated knockdown of HDAC2 reversed the downregulation of such genes, improved synaptic plasticity and eliminated the neurodegeneration-associated cognitive decline observed in the Ck-p25 transgenic mice [27]. Increased HDAC2 levels were further observed in the hippocampus of 5XFAD transgenic mice, which carry five familial mutations associated with Alzheimer's disease (AD), while HDAC2 levels increased in primary hippocampal neuronal cultures after exposure to AD-related neurotoxic insults, i.e. H₂O₂ and A β oligomers [27]. On the other hand, contrasting results have been reported in the human AD brain, where Graff *et al.* reported increased HDAC2 levels in the entorhinal cortex and hippocampus, while Mastroeni *et al.* reported decreased levels of HDAC2 in the entorhinal cortex, when comparing AD patients to non-demented controls [27, 38, 39]. Our findings of increased hippocampal HDAC2 with aging are in line with recent reports of increased HDAC2 levels in the hippocampus of aged (22–23 month-old) rats [40]. Zeng and colleagues further demonstrated that increased HDAC2 and decreased histone acetylation were associated with decreased expression of brain-derived neurotrophic factor (BDNF), age-associated deficits in long-term synaptic plasticity and loss of dendritic spines, while HDAC inhibition or activation of trkB receptors could ameliorate these effects. Age-related decreases in acetylation of histone H3 at lysines 9 and 14, particularly of genes related to neuronal function, has furthermore been reported in the human prefrontal cortex [41]. Moreover, an age-related decrease in acetylation of H4 has been shown in neuronal cell culture studies from wild-type mice, while the opposite pattern was observed in neurons cultured from 3xTg-AD mice [42]. Altogether, these findings suggest a causal contribution of aberrant histone acetylation and increased levels of HDAC2 to age-related changes in brain function. Clearly, such data provide evidence for the role of epigenetic mechanisms on mediating complex gene-environment interactions leading to the development of age-related neurodegeneration [43]. Evidence from the literature, such as the Latent Early-life

Associated Regulation, (LEARN) model paradigm further suggest that these changes might already be primed at early developmental stages of life [44, 45].

Correlation and Colocalization of HDAC2 and 5-mC IR

Previously, our group has reported an age-related increase in levels of the DNA methylation marker 5-mC (which was prevented by CR) [10]. The observed positive correlations between the previously reported 5-mC and HDAC2 IR (as described in the present study) in the CA3 and CA1-2 suggest that the increased levels of HDAC2 might be closely connected to the increased levels of DNA methylation. The lack of correlation between 5-mC and HDAC2 IR in the DG could be explained by the high variation of HDAC2 IR in this subregion and might be associated with the presence of proliferating cells in the DG, the epigenetic programming of which might be different from the aging post-mitotic neurons [9]. The positive correlations between 5-mC and HDAC2 IR may be explained by the fact that HDACs can be recruited by DNA methylation marks and associated methyl-CpG binding proteins in order to induce chromatin remodeling and silence transcription [46, 47].

Three-dimensional image analyses showed that HDAC2 IR colocalized with 5-mC IR. However, HDAC2-IR was also localized in nuclear locations devoid of 5-mC IR. In addition, we observed that HDAC2 IR did not overlap with dense DAPI heterochromatic regions. These findings suggest that HDAC2, first as part of methyl-binding protein complexes, is associated with methylated DNA in the open euchromatin, and second can also act independent of DNA methylation marks, in order to deacetylate histone tails of active genes and induce transcriptional silencing.

Caloric Restriction Prevents Age-Related Increase of HDAC2 IR

Age-related increases of HDAC2 IR in the CA3 and CA1-2 regions were significantly attenuated by CR. 24-month-old CD mice showed a marked increase of HDAC2 IR compared to 12-month-old CD mice, while no significant age-related difference was observed for CR mice. The prevention of this age-dependent effect may be associated with the aging-retardation properties of CR and its impact on gene expression [6, 15, 32]. Moreover, the beneficial effects of CR have been linked with the upregulation of sirtuins, which belong to the class III HDACs [48–50], a finding that further supports a major role for chromatin-modifying enzymes such as HDACs in mediating the effects of CR on gene expression.

SOD1 Overexpression Does Not Affect HDAC2 IR

Transgenic overexpression of SOD1 did not have any effect on HDAC2 IR, which is in line with our previous findings on hippocampal volumes, Dnmt3a IR, 5-mC IR and 5-hmC IR [9–11, 32]. Either SOD1 overexpression does not directly affect these epigenetic marks, or, alternatively, it could be possible that the effects of SOD1 are more pronounced in other tissues than the brain. Then again, it could be speculated that the experimental design, husbandry, position effects promoters used as well as the actual levels of SOD1 over expression and other antioxidants in this model were not sufficient to induce such changes [32, 33, 51].

CONCLUSIONS

The present findings indicate that the level of HDAC2 increases in the aging mouse hippocampus while CR prevents such an effect. Considering the conservative nature of the IR analyses, the biological impact of the observed changes of HDAC2 levels should be further investigated. Additional work is required to clarify whether the activity of HDAC2 is altered during aging and affected by CR. It will also be important to investigate which

specific genes are targeted by HDAC2 and whether their histone acetylation status and gene transcription are altered. As HDACi have been shown to improve age-associated memory impairment and counteract the effects of HDAC2 in mice, additional research is needed to explore the therapeutic potential of HDACi. Importantly, it is to be expected that HDAC inhibition will be accompanied by considerable side effects, given the fundamental role of HDACs in neuronal development, adult neurogenesis and DNA damage response [36, 52–54]. Evidently, more research on the role of epigenetic modifications and their moderators is of crucial importance to decipher fully the role of epigenetic dysregulation in age-associated neurodegeneration and may provide novel targets for prevention or treatment of age-related dysfunction.

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Fig. (1). Hippocampal HDAC2 IR. Representative image of a hippocampal section stained for HDAC2 (bregma level -1.58). The black boxes indicate where the high-magnification images were taken for the analysis. A total of 32 images per animal (i.e. four images in the dentate gyrus, two in the CA3 and two in the CA1-2 regions, at four different bregma levels) were taken for the analysis (see text for more details). Scale bar = $100\ \mu\text{m}$.

Fig. (2). HDAC2 and 5-mC immunofluorescence, and DAPI labeling. Representative high magnification images of a nucleus of a cell in a 12-month-old mouse showing DAPI labeling (**A** and **E**) and fluorescence labeling of 5-methylcytidine (5-mC) (red) in **B** and **F**, and HDAC2 (green) in **C** and **G**, at the corresponding location and focal plane, with merged pictures in **D** and **H**. All images represent one stack taken with the SI-SD system (see text for more details). Note the white arrow indicates the colocalization between HDAC2 and 5-mC (yellow). Images **E–H** show a three-dimensional reconstruction of a representative image stack. The crossing lines indicate the position within the X–Y view at which the Y–Z (E1–H1) and X–Z (E2–H2) views were generated. Scale bar = 10 μm .

Fig. (3).

Representative images of HDAC2 IR. High magnification representative images of the hippocampal regions DG, CA3, and CA1-2. A–C represent a 12-month-old mouse on CD, D–F represent a 24-month-old CD mouse. G–I, represent a 12-month-old mouse on CR, and J–L a 24-month-old CR mouse. Note: An increase in HDAC2 IR is observed from 12 to 24 months, more pronounced in CD mice, while no differences are observed in the CA3 region of 12- and 24-month-old CR mice. The images were taken with a 40× objective. Scale bar = 50 μ m.

Fig. (4).

HDAC2 intensity and surface area. Mean and standard error of means of mean intensity value measurements of HDAC2 IR (A–F). Pooled data from the 4 groups of 12-month-old (white bars) and 24-month-old mice (black bars) are represented separately for DG (A, D), CA3 (B, E), and CA1-2 (C, F). Volume corrections were applied for the surface area occupied by HDAC2 IR measurements. Significant effects ($p < 0.05$ in all cases) in each analysis are indicated with an asterisk in the top right corner of each graph. AU= arbitrary units.

Fig. (5).
HDAC2 and 5-mC correlation analysis. Correlation analysis of HDAC2 and 5-mC IR.
Significant Spearman's correlation coefficients are noted in the bottom right of each graph.