HDAC4 regulates GR signaling Contributes to Stress-Induced Hyperalgesia in the Medial Prefrontal Cortex

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Abstract

“Stress-induced hyperalgesia (SIH)” is a phenomenon that stress can lead to an increase in pain sensitivity. Recent findings showed that epigenetic mechanisms have been known to play fundamental roles in stress and pain. Histone acetylation is an outstanding epigenetic feature that is changed in numerous stress-related disease situations. However, epigenetic mechanism for the control of SIH is not well known. We investigated the effect of histone acetylation on pain hypersensitivity on SPS (single-prolonged stress) + CFA (complete Freund's adjuvant) model. Here, we show that the glucocorticoid receptor (GR) is regulated by histone deacetylases 4 (HDAC4) on stress-induced hyperalgesia and the paw withdrawal threshold in the SPS+CFA group dropped significantly compared with the control group. HDAC4-expressing neurons in the medial prefrontal cortex (mPFC) were increased in the SPS+CFA-exposed group compared with CFA-exposed group. Inhibiting HDAC4 by microinjection of sodium butyrate into the mPFC could disrupting glucocorticoid receptor (GR) signaling pathway, lowered SPS+CFA-caused mechanical allodynia and alleviated anxiety-like behavior. Together, our studies suggest that HDAC inhibitors may involve in the process of stress-induced anxiety-like behavior, amplifying the sensitivity to pain.

Background

Pain experience is not only consisting of sensory-discriminative dimension, cognitive and also affectional processing in the brain [1, 2]. Chronic pain is often endured alongside affective disturbance such as anxiety and depression. Meanwhile affection abnorm could influence pain processing (Nicolson et al. 2009). Clinical observations suggest that stress have been shown to exacerbate pain conditions, recognized as stress-induced hyperalgesia (SIH) [3–5]. The inter-relationship between pain and anxiety has made great challenge for treatmeat of pain. The neurobiological basis for this effect is poorly understood. Stress-induced genetic and epigenetic modifications might be thought to be the underlying cellular mechanism [6].

Recent studies showed that the medial prefrontal cortex (mPFC) play a major role in controlling cognition and emotion such as stress induced-anxiety disorders [7, 8]. Previous studies showed that stress and pain was under regulation by epigenetic mechanisms such as histone modifications [9–11]. Histone deacetylases (HDACs) by removing acetyl groups from lysine side chains regulate protein functions. HDAC4, which belongs to class IIa histone deacetylases family, plays a key role in stress in vitro [12] and inflammation-associated thermal hypersensitivity in vivo [13]. The translocation of HDAC4 from the cytoplasm to the nucleus is induced during stress. Previous studies showed that the effect of HDAC inhibitor MS–275 (a highly selective inhibitor of Class I HDAC) within the mPFC involved in depression-related symptoms [14]. HDAC inhibitors were shown to improve stress-induced memory impairments, and previous studies showed that suppression of HDAC4 by sodium butyrate in the hippocampus abolished stress-induced effects [15].
Stress involved in the hypothalamic–pituitary–adrenal (HPA) axis could influence neurotransmission and synaptic plasticity in the prefrontal cortex. Stress could activate the HPA axis leading to downstream adrenocorticotropic hormone (ACTH) secretion and subsequent secretion of Glucocorticoid (GC) hormones into the circulation [16]. During periods of stress, elevated circulating levels of glucocorticoids are binding to glucocorticoid receptor (GR) [6], which cause ERK1/2/MSK1–Elk–1 pathway activation in order to enhance the impact on epigenetic and gene expression mechanisms [16]. GRs seem to facilitate the activation of MSK1 and Elk–1 by phosphorylated ERK1/2 (pERK1/2) as scaffold. Our previous studies also showed that stress-induced hyperalgesia involved activation of ERK1/2 [17]. However, it is unknown whether chromatin modifications such as histone acetylation regulates GR in the mPFC or GRs act like scaffolds recruiting HDAC contribute to stress-induced hyperalgesia.

In this study, we use a SPS (single-prolonged stress) model [16] or a modified version of SPS [18] to mimic posttraumatic stress disorder (PTSD), injection of complete Freund's adjuvant (CFA) for the persistent pain and a model of SPS+CFA [17]. We examined the interaction between HDAC4 and GR in the mPFC during stress-induced hyperalgesia. Our previous studies demonstrated SPS exacerbated chronic pain. However, whether HDAC4 in mPFC is involved in regulation of GR in the PTSD-pain comorbidity is yet to know. Thus, we inhibited the level of HDAC4 by administering sodium butyrate into the mPFC to determine the contribution of HDAC4 through regulation of GR in the PTSD-pain comorbidity.

**Results**

**Sodium Butyrate treatment attenuates SPS+CFA-induced mechanical hyperalgesia**

The SPS+CFA exposure rats obviously decreased the pain threshold of the hindpaw from day 7. Our previous analysis revealed that PWTs were significantly reduced in the SPS exposure rats (P<0.05), CFA rats after CFA injection (P<0.05), and SPS+CFA exposure rats (P<0.01) from day7 compared to the control rats. The SPS+CFA exposure rats from day 9 showed significantly lower PWT than SPS exposure rats (P<0.05) or CFA exposure rats (P<0.05). Sodium Butyrate chronic treatment in the mPFC increased the PWT in SPS+CFA exposure rats (P<0.05) (Figure 2). The date were not showed that the control group treated with Sodium Butyrate altered the baseline mechanical threshold compared to both the control and the control+vehicle group (P>0.05) and vehicle injection was not significantly different from control group (P>0.05).

**Effects of Sodium Butyrate on anxiety-like behavior induced by SPS and SPS+CFA by EPM**

EPM assessed Anxiety-like behavior induced by SPS and SPS+CFA. The time in open arms (OA time%) and the number of times entry into open arms (OA entries%) are shown. Rats exposed to SPS+CFA or SPS or CFA spent significantly less time in the OA time% and less number in the OA entries% in contrast to the control group (P<0.05) (Figure 3). Observed reduction in OA time% and OA entries% was obviously
changed by Sodium Butyrate chronic treatment in the mPFC of SPS+CFA- (P<0.05), CFA-(P<0.05) and SPS-exposed rats (P<0.05).

**SPS increases GRs expression, and GRs facilitates the activation ERK1/2 (pERK1/2), CREB (pCREB) and Fos**

GR-expressing neurons were observed in the mPFC of rats. Immunofluorescent staining showed that GR-ir was expressed in the nucleus and cytoplasm of the mPFC neurons (Figure 4). We examined GRs levels in rats induced by SPS. SPS induced elevated GRs expression under immunofluorescence and Western Blot analysis (Figure 4). We found that the numbers of the GR-positive neurons were higher in rats of SPS exposure than control rats (P<0.05). Double immunofluorescent staining showed that the colocalization of GR-ir and pERK1/2-ir in the nuclear of mPFC neurons in SPS increased in comparison with the control rats (p<0.05) (Figure 5). Additionally, pERK-ir and Fos-ir, pCREB-ir and Fos-ir neurons were also observed in mPFC neurons in SPS (Figure 6, 7). We counted the number of colocalized pERK-ir and Fos-ir neurons, pCREB-ir and Fos-ir neurons, and found that the numbers of pERK-ir and Fos-ir neurons, pCREB and Fos-ir neurons were higher in rats of SPS exposure than control rats (P<0.05). Next, we examined the protein levels of GR, pERK1/2/tERK1/2, pCREB/CREB and Fos in the mPFC (Figure 8). The Western blot data demonstrated that GR, pERK1/2/tERK1/2, pCREB/CREB and Fos in the mPFC was upregulated in the rats of SPS exposure compared with control rats (P<0.05). These results show that SPS involved the higher levels of GRs and GRs facilitates the activation ERK1/2 (pERK1/2), CREB (pCREB) and Fos.

**SPS+CFA increases HDAC4 and HDAC4 is enriched in GR-ir neurons**

We examined HDAC4 levels in rats induced by CFA, SPS and CFA+SPS. Rats receiving CFA, SPS, or SPS+CFA group showed more HDAC4 levels in contrast to the control group (P < 0.05). There was no change in total H3 expression. We investigated the protein levels of HDAC4. HDAC4 protein expression was increased in the SPS+CFA exposure rats compared with CFA (P < 0.05) or SPS (P < 0.05) exposure rats (Figure 9).

HDAC4-expressing neurons were observed in the mPFC using IHC. Immunofluorescent staining showed that HDAC4-ir was expressed in the nucleus and cytoplasm of the mPFC neurons. IHC labeling result indicated that 95% HDAC4-ir neurons were positive for GR in SPS+CFA group. In addition, HDAC4/GR double labeled neurons in SPS+CFA exposed significantly increased in comparison with CFA (P < 0.05) or SPS (P < 0.05) group (Figure 10).

**Discussion**

Our studies suggested that (1) the PTSD-pain model enhanced the mechanical hypersensitivity, and administration of sodium butyrate into the mPFC blocked the hyperalgesia. (2) the PTSD-pain rats showed anxiety-like behaviors, and administration of sodium butyrate into the mPFC attenuated anxiety-like behaviors. (3) SPS-treated rats induced more expression of GRs, pERK, pCREB and Fos in the mPFC.
It suggests that SPS-induced changes might underline stress-induced hyperalgesia. (4) the PTSD-pain rats produced more HDAC4 changes. And, HDAC4/GR double labeled cells in rats of PTSD-pain model significantly increased in comparison with CFA or SPS group.

Stress is a state of disharmony or threatened homeostasis, which could modulate pain perception, resulting in either analgesia caused by stressor hyperalgesia caused by stress [19]. Acute stress could produce antinociception [20], while chronic stressful stimuli could produce an increase in pain sensitivity [21]. Stressful stimuli could also produce an increase in different type of chronic pain disorder [3–5]. Epidemiological and clinical studies have been shown that PTSD could exacerbate the chronic pain disorder [22]. However, less is known about the mechanic of SIH. Activation of the stress system induces various changes in body systems, including activating the hypothalamic-pituitary-adrenal (HPA) axis, causing the glucocorticoids (GCs) release [23]. Glucocorticoids enter the brain and bind to glucocorticoid receptor (GR), which is expressed in the prefrontal cortex [24]. Our results showed that SPS could induce more GRs expression in the PFC. Glucocorticoid acting via GRs might lead to stimulation of NMDARs, then through Ca\textsuperscript{2+}-CAMKII-ERK1/2 signaling.

Our previous and present studies showed that PTSD upregulated ERK1/2. Meanwhile, our present studies showed that SPS induce more expression of pCREB and Fos. These results suggest that SPS-induced GC-mediated response might through GRs-ERK-CREB-Fos pathway.

Epigenetics is a heritable phenomenon in which the environment can cause lasting changes in gene transcription without alterations in the sequence of the gene. Despite recent findings showed that epigenetics have been known to play a key role in pain and chronic stress-induced behavior [16], whether epigenetics is involved in the stress-induced hyperalgesia remains unknown. Thus, in the present study, we determined the mechanisms of PTSD-pain comorbidity.

Recent findings showed that during stress gene expression in the central nervous system was regulated by epigenetics. The HPA is important and necessary in coordinating both rapid and long-term behavioral, physiological and molecular responses to psychogenic stressors in the brain system, leading to disorder of the neural, endocrine, and immune system and the secretion of glucocorticoids (GCs) [9]. During stress, long-term and rapid, dynamic gene expression is regulated by Epigenetic mechanisms such as histone modifications leading to the gene on or off, which results in changes in protein production. These changes might lead to mechanical hyperalgesia. Epigenetic mechanism consists of histone modifications, DNA methylation and microRNA activity, of which mechanisms, histone modification is the most important in stress and pain. Histone which regulates gene expression includes histone acetylation by histone acetyl transferases (HATs) and histone deacetylation by histone deacetylases (HDACs). Histone proteins are in charge of organizing DNA into chromat in which can exist in a two forms (closed or open state) [16]. Histone acetylation which is associated with active gene transcription for numerous genes studied is the modification most likely to decondense chromatin and expose previously silent genes for transcription [25]. In this study, our morphological data suggested that PTSD-pain animals upregulated HDAC4/GR in the mPFC. It suggested that HDAC4 might regulates GR in the mPFC contributes
to stress-induced hyperalgesia. Meanwhile, behavior results showed that PTSD-pain animals induced anxiety-like behaviors and hyperalgesia, which could be partly reversed by injection with a highly non-selective HDAC inhibitor, Sodium butyrate. Through the above results, we could figure out stress-induced disorder of the neural, endocrine, and immune system might be associated with histone modification. More HDAC4/GR activation producing emotional disorder might be related to “limbically augmented pain syndrome”, which might be the neurobehavioral mechanisms underlying stress-induced hyperalgesia.

Previous results have demonstrated that the epigenetic signal cascade transmission starts with an “epigenator”, such as REST and CREB [26]. Epigenator is a concept which referred to all signals, including environmental cues and intrinsic processes, that end up with the recruitment of an “epigenetic initiator”. Furthermore, the initiator could cause modifications without signals and persist within a neuron with an “epigenetic maintainer”. The epigenetic initiator recruits epigenetic maintainer, and they might act through histone modification and DNA methylation to establish epigenetic patterns [27]. In this study, our data revealed that GRs-ERK-CREB-Fos signaling in SPS might serve as epigenetic initiator. HDAC4 are recruited by GR in SPS+CFA, which might be “epigenetic maintainer”. Anyway, the epigenetic pattern still to be known and more studies should be explored in elucidating the phenomenon.

Previous studies suggested that there was a relationship between emotional disorder and allodynia [1]. PTSD is with increased cortisol levels. In the present study, SPS could lead to emotional disorder by behavior tests and significantly reduced paw withdrawal threshold in SPS+CFA compared with SPS and CFA. Our results further confirmed that emotional disorder induced by SPS decrease the pain threshold and exaggerate nociceptive sensitivity.

Conclusions

In generally, our previous and present results showed that SPS exposure could exacerbate chronic pain induced by CFA. PTSD-induced emotional impairments could cause an increase in mechanical allodynia. Enhanced HDAC4/GR activation might lead to the hyperalgesia in SPS+CFA, and GR-pERK-pCREB-Fos pathway may be involved in this processing.

Methods

Animals

Male Sprague-Dawley rats (weighing 250–300 g, Kai Xue science and technology co. LTD, Shanghai) were housed on a 12-hour light-dark cycle with food and water available ad libitum. All animal procedures were carried out in conformity with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and approved by the Ethics Committee of Animal protection of the 960 Hospital of the PLA (Jinan, P. R. China).
SPS procedures were conducted as previously reported for the PTSD model [28]. CFA injection was conducted as previously in our previous studies [17] for the chronic inflammatory pain model. SPS+CFA model was also conducted as previously in our previous studies [17]. A total of 72 animals were used in the study and randomly assigned to one of 4 groups. Each group included 18 rats, 9 for behavioral tests, 5 for immunohistochemical staining, and 4 for Western blot analysis. Behavioral experiments were done double blindly and started at a fixed time during testing days, and rats were always habituated in the testing room for 15min before behavioral tests.

**Antibodies and drugs**

The following antibodies were used: mouse-anti-HDAC4 (SAB5300452; Sigma, St Louis, MO, USA); rabbit-anti-pERK1/2 (pERK, 12185; CellSignaling, Beverly, MA); rabbit anti-total-ERK 1/2 (tERK, 4695; Cell Signaling); mouse-anti-Fos (AB11959; Abcam, Cambridge, MA); rabbit-anti-GR (AB3578, Abcam); rabbit anti-CREB (AB32515, Abcam); rabbit anti-pCREB (AB32096, Abcam). Rats were injected with 100 mg/kg sodium butyrate or saline daily from 2nd – 8th day by microinjections into the mPFC. Sodium butyrate, a highly non-selective HDAC inhibitor.

**Behavioral Tests**

We used Von Frey filament (Stoelting, Kiel, WI, USA) to evaluate the mechanical allodynia of rats. The rats were covered under inverted plastic boxes (35×35×55 cm) on an elevated mesh floor to test the withdrawal responses threshold of the left hindpaw evoked by mechanical stimuli. The method of paw withdrawal threshold (PWT) was done like we did before [17].

The apparatus used for the elevated plus maze test comprises four equally illuminated plastic arms located 50 cm above the floor, including both open arms and closed arms with tall walls. The rats were released from the central area (10×10 cm), facing a closed arm (Shanghai Mobiledatum Technology Co., Ltd, China). The behavior was recorded with a video camera. The time spent on the open arms and open-arm entries provide the measures.

**Immunohistochemical Staining**

The rats were perfused through the ascending aorta by 250 ml of PBS, followed by 500 ml of 4% paraformaldehyde in 0.1 M PB. Brain was removed immediately, postfixed into the same fixative for 2 h (4°C), and then saturated with 30% sucrose in 0.1 M PB overnight (4°C). The frontal forebrain sections (25 μm thick) were cut on a cryostat (Kryostat1720; Leitz) and collected serially in 6 dishes which were incubated overnight at room temperature with following antibodies: (1) rabbit anti-GR antiserum (1/500) and mouse anti-HDAC4 antiserum (1/200); (2) rabbit anti-pERK antiserum (1/200) and mouse anti-HDAC4 antiserum (1/200); (3) rabbit anti-pERK antiserum
(1/200) and mouse anti-Fos antiserum (1/500); (4) rabbit anti-pCREB antiserum (1/200) and mouse anti-Fos antiserum (1/500). The incubation medium used for antibodies contained 5% (v/v) NFBS, 0.3% (v/v) Triton X–100, 0.05% (w/v) NaN3 and 0.25% (w/v) carrageenan in 0.01 M PBS (PBS-NFBS, pH 7.4). The first, second, third and fourth set of sections was rinsed in 0.01 M PBS (pH 7.4, 3 times) and incubated for 4 h in PBS containing 0.3% Triton X–100 with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1/400; Molecular Probes) and Alexa Fluor 594-conjugated goat anti-mouse IgG (1/400; Molecular Probes). All sections were washed in PBS (3 times). Images were observed using a confocal laser scanning microscope (FV1000 Olympus, Tokyo, Japan).

**Western Blot Analysis**

Rats were sacrificed under deep anesthesia by intraperitoneal injection of sodium pentobarbital (60 mg/kg) in 0.9 % (w/v) saline and the brain was quickly removed. Equal amounts of protein (50 μg) selected from mPFC region was denatured and electrophoresed on an 8% SDS-PAG, and then electroblotted onto a polyvinylidenedifluoride membrane (PVDF, Millipore, Billerica, MA, USA). The membranes were incubated overnight at 4°C with mouse anti-HDAC4 antibody (1/1,000; Sigma), rabbit anti-pERK1/2 antibody (1/1,000; CellSignaling Technology), rabbit anti-total-ERK 1/2 antibody (t-ERK, 1/1,000; Cell Signaling Technology), rabbit anti-total-CREB antibody (t-CREB, 1/1,000; Abcam), rabbit anti-pCREB antibody (1/1,000; Abcam), mouse anti-c-Fos antibody (1/1,000; Abcam), and mouse anti-β-actin antibody (1/1,000; Sigma). Then horseradish peroxidase-conjugated secondary antibodies (anti-rabbit 1/3,000, anti-mouse 1/5,000; AmershamPharmacia Biotech Inc., Piscataway, NJ, USA) were used to incubate the membranes for 1 h at RT. Between respective step, the immunoblots were rinsed with Tris-buffered saline with 0.02 % Tween–20 (TBS-T) 3 times for 10 min. All protein bands were detected and analyzed in the Bio-Rad ChemiDoc Imaging System (Bio-Rad Laboratories Ltd, USA).

**Statistical Analysis**

Behavioral results (EPM), the number of pERK1/2 immunoreactive neurons and the expression of protein pERK1/2, pCREB and Fos were analyzed by one-way analysis of variance (ANOVA). Two-way ANOVA with repeated measurement followed by Students-Newman-Keulsas post hoc was used for Mechanical allodynia results and differences between groups. The data are all expressed as means±S. E. M. The tests were two sided. P<0.05 was defined as statistical significance.

**Declarations**

**Abbreviations**

SIH: Stress-induced hyperalgesia; SPS: single-prolonged stress; CFA: complete Freund’s adjuvant; GR: glucocorticoid receptor; HDAC4: histone deacetylases 4; mPFC: medial prefrontal cortex; HPA:
Ethics and Consent to participate

All animal procedures were carried out in conformity with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and approved by the Ethics Committee of Animal protection of the 960 Hospital of the PLA (Jinan, P. R. China). This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors’ contributions

QJ conceived the study. QJ wrote the main manuscript text. ZL prepared figure 1–5. CC prepared figures 6–10. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Ethics and Consent to participate
Ethical approval

All animal procedures were carried out in conformity with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and approved by the Ethics Committee of Animal protection of the 960 Hospital of the PLA (Jinan, P. R. China). This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

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**Figures**

![Experimental design diagram](image)

**Figure 1**

Experimental design showed that the behavioral tests done, the time course of SPS exposure, time course for CFA injections and the duration time for sodium butyrate administration. SPS single-prolonged stress, CFA complete Freund’s adjuvant, PWT paw withdrawal threshold, EPM elevated plus maze.
Figure 2

Effect of CFA+SPS and Sodium Butyrate injection on mechanical hyperalgesia. Von Frey tests showed that SPS+CFA exposure rats had significantly lower mechanical hyperalgesia. Compared with the control rats, the PWT was decreased in the injured hindpaw of the SPS+CFA exposure rats. Sodium Butyrate reversed the PWT reduction (* P<0.05, vs the SPS+CFA group; #P<0.01, vs the control group).
Figure 3

A The percentage of open-arm time (open arm (OA) time/total time) was showed in this histogram in different groups in the EPM test (*P<0.05, vs the no Sodium Butytate CFA group; ** P<0.05, vs the no Sodium Butytate SPS group;*** P<0.05, vs the no Sodium ButytateSPS+ CFA group); B The percentage of open-arm entries (open arm (OA) entries/total entries) was showed in this histogram in different groups in the EPM test (*P<0.05, vs the no Sodium Butytate CFA group; ** P<0.05, vs the no Sodium Butytate SPS group;*** P<0.05, vs the no Sodium ButytateSPS+ CFA group).
Figure 4

Immunofluorescent double labeling between the GR-LI neurons and NeuN in the mPFC by SPS. GR-LI was expressed in the nucleus and cytoplasm of the mPFC (A-C, A'-C'). D showing the statistical analysis of GR/NeuN double-labeled neurons in total NeuN-labeled neurons (*P<0.05, vs the control group).

Figure 5
Immunofluorescent double labelling between the GR-LI neurons and pERK-LI neurons in the mPFC by SPS (A-C, A’-C’). D showing the statistical results of the proportion of GR/pERK double-labeled neurons (*P<0.05, vs the control group).

**Figure 6**

Immunofluorescent double labeling between the Fos-LI neurons and pERK-LI neurons in the mPFC by SPS (A-C, A’-C’). D showing the statistical analysis of the proportion of Fos/pERK double-labeled neurons (*P<0.05, vs control group).
Figure 7

Immunofluorescent double labelling between the Fos-LI neurons and pCREB-LI neurons in the mPFC by SPS (A-C, A’-C’). D showing the statistical analysis of the proportion of Fos/pCREB double-labeled neurons (*P<0.05, vs control group).
Figure 8

A Immunoblots of GR, pERK1/2/tERK1/2, pCREB/tCREB and Fos in the mPFC. B, C, D, E Densitometry analysis of Western blot bands of GR, pERK1/2/tERK1/2, pCREB/tCREB and Fos. Compared to the control group, the protein of GR, pERK1/2/tERK1/2, pCREB/tCREB and Fos was up in SPS exposure rats (*P<0.05, vs control group).
Figure 9

A Immunoblots of HDAC4, H3 in the mPFC. B Densitometry analysis of Western blot bands of HDAC4/H3. The active protein level of HDAC4/H3 was up in SPS+CFA exposure rats (*P<0.05, vs the control group; # P<0.05, vs the SPS group; ▲P<0.05, vs the CFA group).
Figure 10

Immunofluorescent staining of HDAC4/GR in the mPFC in each group. A-C Control; A’-C’ CFA; A”-C” SPS. A’’-C” SPS+CFA. Scale bars=100 μm. D Comparison of the number of HDAC4/GR immunoreactive cells indifferent groups (*P<0.05, vs the control; # P<0.05, vs the SPS; ▲ P<0.05, vs the CFA).

Supplementary Files

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