Repression of contextual fear memory induced by isoflurane is accompanied by reduction in histone acetylation and rescued by sodium butyrate

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Editor’s key points
- The role of histone acetylation in isoflurane-induced amnesia was studied.
- Repression of contextual fear conditioning by isoflurane correlated with reduced histone acetylation and cFos expression.
- A histone deacetylase inhibitor prevented both the reduction in histone acetylation and the repression of fear conditioning by isoflurane.
- Changes in chromatin modification and gene expression correlate with isoflurane-induced amnesia.

Background. Isoflurane produces amnesia in mice during contextual fear conditioning (CFC) trials. Histone acetylation is a form of chromatin modification involved in the transcriptional regulation underlying memory formation. We investigated whether isoflurane-induced repression of contextual fear memory is related to altered histone acetylation in the hippocampus, and whether it can be rescued by the histone deacetylases inhibitor sodium butyrate (SB).

Methods. Adult C57BL/6 mice were chronically given intraperitoneal injections of SB or vehicle for 28 days. Immediately before CFC training, the mice were exposed to isoflurane or air for 30 min and CFC testing was performed the next day. Hippocampal histone acetylation was analysed 1 h after CFC training. c-Fos, an immediate early gene (IEG) suggested to participate in learning and memory formation, was also investigated at the same timepoint.

Results. Mice exposed to isoflurane showed a reduction in freezing time during the CFC test. These mice also exhibited reduced hippocampal H3K14, H4K5, and H4K12 acetylation 1 h after CFC training, and also decreased c-Fos expression. All of these changes were attenuated in isoflurane-exposed mice that were chronically treated with SB.

Conclusions. Isoflurane suppresses histone acetylation and down-regulates c-Fos gene expression in CA1 of the hippocampus after CFC training. These changes are associated with isoflurane-induced amnesia. The HDAC inhibitor SB prevented repressed contextual fear memory, presumably by promoting histone acetylation and histone acetylation-mediated gene expression in response to CFC training.

Keywords: amnesia; anaesthetics volatile, isoflurane; epigenetics, acetylation; hippocampus; mice; proto-oncogene proteins c-Fos; sodium butyrate

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Animal studies have demonstrated that low inhaled concentrations of isoflurane suppress learning and memory in contextual fear conditioning (CFC).1–3 The contextual fear memories of animals are gradually repressed when isoflurane inhaled concentration increases from 0% to 0.5% before CFC training. In CFC trials, animals are exposed to a particular neutral context paired with an aversive electric shock. If the context is associated with electric stimuli, fearful responses are observed when animals are placed in that context again. Lesion studies in animals have demonstrated that the hippocampus play a critical role in CFC.4–5 and CFC has become a common method for studying hippocampus-dependent associative memory.

Alterations in gene expression within seconds or hours after learning are thought to be required for long-term memory formation. There is a wide range of mechanisms underlying regulation of gene expression; modification of chromatin structure has particular significance for gene transcription.6 Histone acetylation can relax condensed chromatin and result in greater gene transcription.7–9 Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Recently, a study of hippocampal memory formation indicated that enhanced histone acetylation due to selective recruitment of HDAC inhibitors can facilitate formation of long-term memory.10 This result has been confirmed in several studies11–13 and suggests that histone acetylation-related chromatin modification plays an important role in the transcriptional regulation underlying memory formation.

We hypothesized that gene expression regulation via histone acetylation is involved in isoflurane-induced repression of CFC memory and that HDAC inhibitors could rescue this...
effect. To examine this, we investigated the influence of isoflurane on contextual fear memory and hippocampal histone acetylation in response to CFC training. We also determined whether these effects can be attenuated by systemic administration of sodium butyrate (SB), which potently inhibits Class I HDAC, including HDAC1, 2, 3, and 8. In addition, we investigated expression of c-Fos, an immediate early gene (IEG) that has been used as a marker of neuronal activation, and assessed its correlation with changes in hippocampal histone acetylation.

**Methods**

**Animals**

A total of 164 adult male C57BL/6 mice (3 months old, 20–25 g), obtained from the experimental animal centre of Central South University, were used. Animals were housed in cages and allowed access to food and water ad libitum. Cages were kept in a 12 h light/dark cycle at a room temperature of 24 (1) °C. All procedures were performed with the approval of the animal ethics committee of Xiangya Hospital Central South University and according to local policies. The relevant aspects of the ARRIVE guidelines were adhered to as appropriate.

**SB administration and isoflurane delivery**

SB was dissolved in saline as vehicle and chronic treatment was administered as 1.2 g kg⁻¹ once-daily intraperitoneal injections for 28 days (Fig. 1A). The control group animals received saline injections. All injections were given between 7:00 and 9:00 p.m.

For isoflurane delivery, mice were kept in a gas-proof box (40 × 35 × 25 cm, with a gas inlet and outlet) for 30 min. The box was filled with the target concentration of isoflurane (0.4 vol% isoflurane + 30% O₂, 1 litre min⁻¹) through the inlet, and the outlet gas concentration was monitored by Capnomac Ultima anaesthesia monitor (Daetex-Ohmeda/GE Healthcare, Wauwatosa, WI, USA). After exposure to isoflurane, each animal was quickly transferred into the CFC training chamber which was filled with the same concentration of isoflurane. For control group animals, the gas-proof case and training chamber were filled with oxygen-enriched air (30% O₂, 1 litre min⁻¹).

**Behavioural procedure**

Animals were handled for 4 days, and on the day of experiments, they were transported to the laboratory at least 2 h before isoflurane delivery and CFC training. For CFC, the transparent Plexiglas training chamber (40 × 30 × 26 cm) was placed into a soundproof box (75 × 60 × 45 cm) with a camera fixed on the top. The video of each animal during CFC was captured by ANY-maze software (Stoelting Co, Wood Dale, IL, USA.). The floor of training chamber had 28 iron bars which could provide electric shock. Mice were kept in the training chamber about 5 min for CFC training. In the first 2 s of the 5th minute, mice received an electric footshock of 1.0 mA for 2 s. ‘Freezing’ behaviour was measured with ANY-maze software. A freezing score of <30 is considered to enter the freezing state and >40 was defined to be off freezing state. The quantification of freezing time during CFC training started at the 2nd minute and was terminated before the footshock was given. For assessment of contextual fear memory, freezing time at 24 h post-training was measured for three consecutive minutes in the training chamber.

Isoflurane is an inhaled anaesthetic that supplies several essential elements of anaesthesia: hypnosis, immobility, and amnesia. Previous studies have shown that the concentrations of isoflurane required to suppress contextual fear memory are 0.3–0.5 vol%. We used a 0.4 vol% isoflurane for our memory-repression model in view of the above-described studies and our preliminary experiments because this concentration induces sufficient amnesia with minimal immobility (data not shown). Mice exposed to 0.4 vol% isoflurane for 30 min showed a reduction in freezing during the CFC test, whereas freezing time of each group during CFC training was similar, regardless of whether or not the mice were exposed to isoflurane. In addition, when a single 1.0 mA footshock was given, all mice, including the groups exposed to isoflurane for 30 min, showed visible nociceptive reflexive behaviours, including screaming, jumping, and withdrawal (data not shown).

To investigate whether exposure to isoflurane itself has an influence on freezing behaviour in response to novel context, mice exposed to isoflurane or air for 30 min underwent the same training procedure as above, but did not receive a footshock during a mock training session.

To ensure the objectivity of the study, the researchers who were in charge of CFC test, immunohistochemistry and western-blot analysis which were described in following sections did not know the treatment and training program of each group until unblinding.

**Tissue preparation and immunohistochemistry**

At 1 h after CFC training, mice were killed by rapid cervical dislocation. Isolated brains were fixed using 4% paraformaldehyde for 24 h at 4 °C. After cryoprotection in 30% sucrose–PBS for 2 days at 4 °C, brains were embedded in 30% sucrose and stored at 4 °C. Tissue was cryo-sectioned at 50 μm thick and the hippocampus processed for immunohistochemistry. Tissue sections were incubated successively with primary antibody (12 h, 4 °C), serum (10 min, room temperature), and biotin-conjugated secondary antibodies (30 min, 37 °C). The primary antibodies used were: anti-acetyl H3K9, #9671, 1:200, Cell Signaling Technology, Danvers, MA, USA; anti-acetyl H3K14, #4318, 1:200, Cell Signaling Technology; anti-acetyl H4K5, #9672, 1:1600, Cell Signaling Technology; anti-acetyl H4K12, SC-34266, 1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA; anti-c-Fos, #2250, 1:200, Cell Signaling Technology. The tissue sections were then incubated with streptavidin–horseradish peroxidase complex (Maixin, Fuzhou, China) for 10 min. Immunoreactivity was seen by incubating the sections with 3, 3 ′-diaminobenzidine (DAB, Maixin) for 2–10 min. Sections for c-Fos analysis were counterstained with haematoxylin. Finally, sections were dehydrated, washed, and fixed onto gelatin-coated slides (China National Medicines, Shanghai, China). The hippocampal CA1, CA3, and DG regions of all
sections were observed under a Leica DM5000B microscope (Leica Microsystems CMS GmBH, Wetzlar, Germany). In order to quantify histone acetylation and c-Fos expression, we examined the relative optical densities (OD) of neuronal histone acetylation immunoreactivity and the number of c-Fos positive cells in these three subregions using ImageJ software (version 1.46 for Windows, National Institutes of Health, Bethesda, MD, USA). The mean OD value of the immunoreactive neurones was measured on transformed digital images after precise delineation of region of interest at 200×. The mean OD value of background staining was obtained in hippocampal stratum radiatum, where almost no histone acetylation-positive reaction products was observed, and subtracted from all measures of immunoreactive neurones before statistical analysis. c-Fos protein was quantified at 400× by positive cells counting because of the low level of expression. As a negative control, tissue sections were subjected to the same procedure, but the primary antibody was omitted, which resulted in no positive immunoreactivity.

Tissue extraction and western blot analysis

At 1 h after CFC training, six mice from each group were killed by cervical dislocation. Each brain was quickly removed at 4°C and placed into oxygenated ice-cold cutting saline [containing the following (in mm): 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH2PO4, 28 NaHCO3, 0.5 CaCl2, 2 MgCl2, 5 glucose, 0.6 sodium ascorbate] before isolation of the CA1 region. From transverse hippocampal slices, the CA1 regions were dissected with a needle under a dissecting microscope, then the isolated CA1 tissues were snap-frozen in nitrogen. The collected tissues were homogenized in RIPA buffer (Applygen Technologies Inc., Beijing, China) for 30 min at 4°C. After centrifugation (9 000 g, 5 min, 4°C), supernatants were gathered and cryopreserved for c-Fos analysis. The debris was collected and resuspended in 0.2 M HCl overnight at 4°C for acid extraction of histones. The extracts were centrifuged at 14 000 g for 10 min. Supernatants were aspirated and cryopreserved for subsequent immunoblot analysis. A bicinchoninic acid method (Well-biology Technologies Inc., Changsha, China) was used to determine protein concentrations. Aliquots of sample were then normalized to 2 mg ml⁻¹. Equal amounts of protein were loaded and then separated by sodium dodecyl sulphate–polyacrylamide electrophoresis. Proteins in the gel were then transferred onto a membrane of polyvinylidene difluoride. After blocking with 5% non-fat dry milk, membranes were incubated overnight at 4°C with the following primary antibodies: anti-actin, ab34731, 1:3000, Abcam, Cambridge, UK; anti-H3 histone, ab1791, 1:3000, Abcam; anti-H4 histone, #2935, 1:1000, Cell Signaling Technology; anti-acetyl H3K9, #9671, 1:1000, Cell Signaling Technology; anti-acetyl H3K14, #4318, 1:1000, Cell Signaling Technology; anti-acetyl H4K5, #9672, 1:1000, Cell Signaling Technology; anti-acetyl histone H4K12, SC-34266, 1:200, Santa Cruz Biotechnology; anti-c-Fos, #2250, 1:1000, Cell Signaling Technology.
Membranes were then incubated for 45–60 min at 21 (2) °C using a secondary antibody (1:3000, Proteintech, Chicago, IL, USA). Bands were seen using SuperECL Plus reagents (Thermo Fisher Scientific, Waltham, MA, USA). Software Quantity One 4.62 (Bio-Rad, Hercules, CA, USA) was used to quantify the relative band densities of acetyl-histone and c-Fos protein.

For the purpose of investigating the effect of isoflurane on hippocampal histone acetylation and c-Fos expression alone, two additional control groups were added. Mice in the first control group (naive group) were transported to the laboratory and housed in the gas-delivery chamber for 4 days before air or isoflurane exposure, and were killed 1 h after gas delivery without receiving CFC. Another control group received the experimental manipulations of mock training as described above, with killing by cervical dislocation 1 h after training. The tissue preparation and western blot analysis of histone acetylation and c-Fos protein were performed with the same methods outlined above.

### Statistical analysis

Mice were assigned sequence numbers by weight, and randomly placed into groups using a random number table. Investigators conducting the CFC test, immunohistochemistry, and western blot analysis were blinded to group assignments. Statistical analyses were processed by software SPSS (version 13.0, SPSS, Chicago, IL, USA). Two-way analysis of variance (ANOVA) with repeated measures was used to compare the proportions of freezing time in CFC trial among all groups. The two factors were air/ISO and vehicle/SB. Western blotting and immunohistochemistry data were analysed by one-way ANOVA with the Student–Newman–Keuls (SNK) or Dunnett tests. A P-value of <0.05 significance level was chosen in all cases.

### Results

During CFC training, mice were subjected to a new context paired with a footshock. In this training process, mice show less freezing behaviour during the period of pre-footshock and no significant difference among all groups was detected, \((F_{3,52}=0.991, P=0.404)\). When re-exposed to the CFC context the next day, mice exposed to 0.4 vol% isoflurane for 30 min showed a reduction in the proportion of time spent freezing compared with two air-exposed groups (all \(P<0.01\)). Systemic administration of SB increased freezing behaviour during the CFC test in mice exposed to 0.4 vol% isoflurane for 30 min immediately before CFC training \((P=0.002, \text{Fig. 1e})\). For mock training, no difference in freezing time was detected between the air and isoflurane groups, during the mock training session or if re-exposed to same context 24 h later (all \(P>0.05\), Fig. 1c). In addition, mice exhibited no increase in freezing time 24 h after the mock training (all \(P>0.05\), Fig. 1c).

In the immunoblotting studies, mock training increased H3K9, H3K14, and H4K12 acetylation (all \(P<0.05\)) and CFC training increased the H3K9, H3K14, H4K5, and H4K12 acetylation in hippocampal CA1 region (all \(P<0.01\)) compared with naive (home cage) animals. When mice were kept in their home cage, exposure to isoflurane did not affect histone
acetylation or c-Fos expression in hippocampal CA1 region (all
P > 0.05). Similarly, there was no significant difference between
air/isoflurane-exposed groups in histone acetylation and c-Fos
protein expression after mock training (all P > 0.05). Isoflurane-induced reductions in H3K14, H4K5, and H4K12
acetylation and c-Fos protein expression in the CA1 region
were only detected at 1 h after CFC training (all P < 0.01, Fig. 2).

At 1 h post-CFC training, we observed that H3K14 and
H4K12 acetylation was significantly decreased in the hippo-
campal CA1 regions of isoflurane-exposed mice by semi-
quantitative immunocytochemistry (all P = 0.05, Figs 3a and
4a). For H4K5 acetylation, the reductions caused by isoflurane
were observed in both CA1 and DG regions (all P < 0.05,
Fig. 4c). Systemic administration of SB ameliorated the reduc-
tions in H3K14, H4K5, and H4K12 acetylation in related subre-
gions of isoflurane-exposed mice 1 h after CFC training (all
P < 0.05, Figs 3 and 4). In the immunoblotting analysis, expos-
ure to isoflurane reduced in H3K14, H4K5, and H4K12 acetyl-
ation in hippocampal CA1 region 1 h after CFC training (all
P < 0.01, Figs 3r and 4r). Systemic administration of SB pro-
moted H3K14, H4K5, and H4K12 acetylation in the CA1
region only when mice were exposed to isoflurane for 30 min
immediately before CFC training (all P < 0.05, Figs 3r and 4r).
Notably, intraperitoneal administration of SB did not have
any effect on hippocampal H3K9, H3K14, H4K5, or H4K12
acetylation in air-exposed mice (all P > 0.05, Figs 3r and 4r).

The number of c-Fos-positive cells was significantly decreased
in the hippocampal CA1 region of isoflurane-exposed mice 1 h
after CFC training (P = 0.001, Fig. 5a). Chronic intraperitoneal
administration of SB up-regulated c-Fos expression in the
CA1 region of isoflurane-exposed mice (P = 0.035) but had no
effect in air-exposed mice (P = 0.999, Fig. 5a). In addition, there
were no significant differences in c-Fos-positive cell numbers
in region CA3 and DG between groups (all P > 0.05, Fig. 5a).
Immunoblotting c-Fos expression showed that the lowest level
was observed of mice exposed to isoflurane immediately
before CFC training. Among mice exposed to isoflurane,
the group with chronic intraperitoneal SB administration showed
a higher level of hippocampal c-Fos protein than the group that
received vehicle (P = 0.013, Fig. 5c).

Discussion
During long-term memory formation, memory-associated sig-
nalling begins when N-methyl-D-aspartate (NMDA) glutamate
receptors and non-NMDA-type glutamate receptors located
on the cell membrane are activated. Activation of glutamate-
gated channels leads to transmembrane ion flow, which
initiates downstream signalling pathways that converge to ac-
tivate several transcription factors/co-activators that the gene
expression underlying long-term memory formation. CBP [cAMP-responsive element binding protein (CREB) binding
protein] is a transcription co-activator with HAT activity and is
up-regulated during long-term memory formation. Histone
acetylation mediated by CBP is a rate-limiting step in transcrip-
tional activation underlying memory stabilization. We found
that isoflurane repressed contextual fear memory, which was
accompanied by reductions in hippocampal H3K14, H4K5,
and H4K12 acetylation. The existing data suggest that the
anaesthetic effects of isoflurane are attributed to a combined
effect on the potentiation of γ-aminobutyric acid (GABA)-gated
channels and inhibition of glutamate-mediated synapse trans-
mition. Sonner and colleagues found that the capability of
isoflurane to induce amnesia does not require GABA receptor ac-
tivation, implying that inhibition of glutamate-gated channels
might play a key role in isoflurane-induced amnesia. The
inhaled anaesthetics can depress both NMDA and non-NMDA
glutamate receptors, although the former are more sensitive
to isoflurane. Inhibition of glutamate-gated receptors represses
post-synaptic transmission of memory-associated signalling
that might affect activation of CBP and ultimately result in
dehesd histone acetylation.

We assessed the acetylation of several histone residues
(H3K9, H3K14, H4K5, and H4K12) shown to be important
for learning, memory, and synaptic plasticity at 1 h after
CFC training because a previous study demonstrated that
acetylation of these histone residues increased by CFC training
almost reached a peak at this timepoint. We showed that there
were isoflurane-induced reductions in acetylation of H3K14,
H4K5, and H4K12 in response to CFC training task without sig-
nificant changes in H3K9 acetylation. These results provide the
first evidence that isoflurane-induced amnesia might involve
hippocampal histone acetylation. Intriguingly, acetylation
of H3K9, which was demonstrated to be associated with cogni-
tive dysfunction caused by brain iron overload mediated by
HDAC1 and plays an important role during fear extinction,
does not appear to be related to isoflurane-induced amnesia.

It is interesting that isoflurane-induced changes in hippocam-
pal histone acetylation 1 h after CFC training occurred mainly
in the CA1 region. CA1 is the major subfield that has substantial
reciprocal connections with the amygdala, which might be
involved in formation of contextual fear memory. Likewise,
several functional neuronal imaging studies found learning-related alterations occurred mainly in CA1 instead of
other subregions. Because we assessed histone acetylation
at a single timepoint after CFC training, there is a possibility
that changes in histone acetylation may be overlooked in regions
with more rapid but transient response to CFC training such as
DG or CA3.

Isoflurane showed an inhibitory effect on hippocampal
histone acetylation 1 h after CFC training, but failed to change
the levels of histone acetylation when mice were kept in their
home cage. These results suggest that isoflurane repressed con-
textual fear memory by repressing the activation of histone
acetylation in response to learning but did not affect baseline
levels of histone acetylation under naive condition. In addition,
mock training was also able to increase histone acetylation.
In mock training, histone acetylation in the CA1 region was
induced by novel context, even when not associated with fear
learning. However, an interesting complexity to our study is
that isoflurane failed to change the levels of histone acetylation
1 h after mock training. A likely explanation is that the mock
training group was actually exposed to two novel contexts in suc-
cession and the first novel context, the gas-delivery chamber,
might have initiated the non-associative learning-related signalling pathway before the mice inhaled enough isoflurane to induce amnesia. Another possibility is that the influence of 0.4% isoflurane on novel context exposure-induced histone acetylation and c-Fos expression is negligible and the different effects of isoflurane between CFC learning and mock training are due to administration of footshock.

Histone acetylation catalysed by HATs alters chromatin structure and allows recruitment of transcription factors/co-activators and initiation of transcription procedure, whereas HDACs-mediated histone deacetylation is commonly considered to represent a transcriptional repression. Chromatin remodelling via histone acetylation is reversible and dynamic, so the experiential stimuli can quickly induce the changes of
epigenome, which affect the expression of memory-associated genes, such as c-Fos, that is necessary to the synaptic plasticity and suggested to participate in learning and memory formation.\textsuperscript{34,35} c-Fos expression is regulated by histone acetylation,\textsuperscript{36,37} and hippocampal c-Fos expression increases after CFC training.\textsuperscript{32,38} We found hippocampal that isoflurane suppressed c-Fos expression in CA1 neurones simultaneously with decreases in H3K14, H4K5, and H4K12 acetylation. Acetylation of H3K14, H4K5, and H4K12 seem to be associated with downstream changes of the IEG product c-Fos, specifically in the hippocampal CA1 region. c-Fos might activate secondary waves of transcription that are important for memory formation.\textsuperscript{34}

In previous studies, HDAC inhibitors such as SB or trichostatin A were reported to increase long-term potentiation at
Schaffer collaterals in hippocampal CA1 region and rescue memory deficits in both aged and mutant mice. SB has been demonstrated to increase histone acetylation in neuronal cells and to be potent in the treatment of depression and Huntington's disease mouse models. SB can induce short-lasting acetylation of histones in the liver and hippocampus. Nevertheless, we did not administer SB acutely, a short period of hypoactivity (about 30 min) can be observed after intraperitoneal injection of SB (data not shown). If SB was administered before isoflurane exposure or CFC training, we could not be sure if there was a synergistic effect between isoflurane and SB that would affect our behavioural observation. In addition, if the mice were injected immediately after CFC training, the accompanying noxious stimuli so near to footshock stimuli might disturb the link between context and footshock. We used chronic systemic administration of SB based on previous studies. It should be noted that because we did not assess the expression of other memory-related genes or perform genome-wide analysis of transcription at various timepoints following CFC, it is possible that the SB-induced changes in expression of other memory-related and histone acetylation-regulated genes are

Fig 5 (A) Representative immunocytochemistry images of c-Fos in hippocampal region CA1, CA3, and DG of each group 1 h after CFC training. Hippocampal neuronal nuclei were stained purple with haematoxylin, nuclear c-Fos protein was stained brown. (B) c-Fos-positive cell counts. Mice exposed to isoflurane exhibited a reduction in c-Fos positive CA1 neurones 1 h after CFC training. Chronic systemic SB administration rescued isoflurane-induced reduction in c-fos expression. No difference in c-Fos between the groups was observed in the CA3 and DG region. *P<0.05, **P<0.01 compared with the ISO+vehicle group. Mean (±SD) shown. (C) c-Fos protein levels in CA1 determined via immunoblotting 1 h after CFC training. c-Fos protein expression was measured by the ratio of c-Fos/β-actin, *P<0.05, **P<0.01 vs ISO+vehicle group (n=6). Results were normalized against air+vehicle group, mean (±SD) shown.
induced reductions in histone acetylation result in reduced c-Fos expression in histone acetylation. These observations show that isoflurane was inhibited 1 h after CFC training when mice were exposed to air might be due to a ‘ceiling effect’ caused by a ‘strong’ footshock of 1.0 mA.\(^{45}\) For air-exposed mice, there might also be a ‘ceiling effect’ in the activations of histone acetylation following CFC learning and hence SB was shown to have no effect when the activations of histone acetylation reached to their greatest extent after optimal associative learning and memory.

In summary, our results indicate the repression of contextual fear memory induced by isoflurane is caused by reduced histone acetylation in the hippocampus. c-Fos expression was inhibited 1 h after CFC training when mice were exposed to 0.4% isoflurane, which is in line with the observed changes in histone acetylation. These observations show that isoflurane-induced reductions in histone acetylation result in reduced c-Fos expression appear to contribute to isoflurane-induced amnesia, which can be rescued by HDAC inhibition.

**Authors’ contributions**


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**Declaration of interest**

None declared.

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