Contribution of histone acetylation to the serotonin-mediated long-term synaptic plasticity in terrestrial snails

Alena B. Zuzina
Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia

Pavel M. Balaban (pmbalaban@gmail.com)
Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia

Research Article

Keywords: serotonin, long-term potentiation, histone acetylation, epigenetics, Helix

Posted Date: April 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1528899/v1

Abstract

Serotonin plays a decisive role in long-term synaptic plasticity and long-term memory in mollusks. Previously, we demonstrated that histone acetylation is a critical regulatory mechanism of long-term memory in terrestrial snail. At the behavioral level, many studies were done in Helix to elucidate the role of histone acetylation and serotonin. However, the impact of histone acetylation on long-term potentiation of synaptic efficiency in electrophysiological studies in Helix has been studied only in one paper. Here we investigated effects of serotonin, histone deacetylases inhibitors sodium butyrate and trichostatin A, and a serotonergic receptor inhibitor methiothepin on long-term potentiation of synaptic responses in vitro. We demonstrated that long-term potentiation was suppressed by methiothepin but rescued by co-application of histone deacetylase inhibitors sodium butyrate or trichostatin A. Increased histone acetylation compensated the loss of serotonergic activation. We showed that single serotonin application in combination with histone deacetylase blockade could mimic the effect of repeated serotonin applications and be enough for sustained long-lasting synaptic changes. The data obtained demonstrated that histone deacetylases blockade ameliorated deficits in synaptic plasticity induced by different paradigms (methiothepin treatment, the weak training protocol with single application of serotonin), suggesting that histone acetylation contributes to the serotonin-mediated synaptic plasticity.

Introduction

According to modern concepts, serotonin plays a decisive role in the development and maintenance of long-term synaptic plasticity and long-term memory in mollusks (Kandel and Schwartz 1982; Balaban et al. 1987; Balaban and Bravarenko 1993; Balaban 2002; Alberini and Kandel 2014; Balaban et al. 2016; Deryabina et al. 2018), but the molecular mechanism of its action still remains unclear.

Recently, experimental data on the link between serotonin and histone acetylation, its role in cognitive functions appeared in literature (Zhu et al. 2021). It should be noted that the histone acetylation itself plays an important role in synaptic plasticity and memory in invertebrates and vertebrates and acts as an epigenetic controller (Vecsey et al. 2007; Chen et al. 2014; Barichello et al. 2015; Villain et al. 2016; Hu et al. 2018; Campbell and Wood 2019; Zuzina et al. 2020; Kolotova et al. 2021). The inhibition of histone deacetylases was shown to enhance the function of serotoninergic neurons in organotypic raphe slice culture in rodents (Asaoka et al. 2015). Agudelo et al. (2012) demonstrated the link between 5-HT3, serotonin, and histone deacetylases and histone acetyltransferases in alcohol dependence. Another study has indicated that inhibition of histone deacetylases significantly upregulated the expression of serotonin transporter (Bence et al. 2011). Activation of the 5-HT2A receptor by the endogenous serotonin repressed the promoter activity of the histone deacetylase 2 gene in mice (Holloway and González-Maeso 2015). The link between serotonin and histone acetylation was demonstrated in *Aplysia californica*: administration of serotonin, providing long-term potentiation (LTP) of synaptic strength, increased the acetylation of histone H3K14 and histone H4K8. Moreover, authors explored the chromatin structure in *Aplysia* neuronal cultures in the context of learning-related synaptic plasticity. Chromatin

immunoprecipitation assays showed that serotonin induced activity of the downstream gene C/EBP by activating CREB1, which recruits CBP for histone acetylation (Guan et al. 2002).

The level of histone acetylation is determined by two groups of enzymes with opposite activity – histone deacetylases (HDACs) and histone acetyltransferases. The use of histone deacetylase inhibitors (HDACis) is effective for studying the role of histone acetylation in regulation of the synaptic plasticity (Peixoto and Abel 2013; Penney and Tsai 2014). In a number of studies, it was shown that the HDACis influence the long-term changes in efficiency of synaptic transmission in cellular and behavioral models (Levenson et al. 2004; Lattal et al. 2007; Federman et al. 2009; Stefanko et al. 2009; Federman et al. 2012; Chen et al. 2014). At the behavioral level, several studies were done in *Helix* to elucidate the role of histone acetylation and effects of serotonin. In our previous studies, we have reported that serotonin precursor 5-hydroxytryptophan, as well as HDACis, sodium butyrate (NaB) and trichostatin A (TSA), restored the impaired memory (Zuzina et al. 2019, 2020). However, the effect of histone deacetylase inhibition on the LTP of synaptic responses in electrophysiological studies in *Helix* was demonstrated previously only once, without investigation of serotonin effects (Kolotova et al. 2021).

Given the importance of histone acetylation in synaptic plasticity, a putative serotonin-histone acetylation connection, and the lack of knowledge regarding the contribution of histone acetylation in synaptic plasticity in *Helix*, we conducted a novel research involving serotonin, HDACis, and nonselective antagonist of serotonergic receptors methiothepin (MET) to investigate the role of histone acetylation in LTP. The data obtained clearly demonstrated that HDAC inhibition ameliorated deficits in synaptic potentiation induced by different paradigms (MET treatment, the weak training protocol with single serotonin application), suggesting that histone acetylation mediates the fundamental processes underlying serotonin-mediated synaptic plasticity.

Materials And Methods

Animals

Experiments were carried out in adult *Helix lucorum taurica L.*(Crimea population). All animals for these experiments were comparable in weight (15g±5). All animals were kept in terraria at temperature 22 ± 2°C, at a 12:12 light/dark cycle. The snails were kept in an active state at least 2 weeks before the experiment in a wet environment and were fed *ad libitum*with cabbage and carrots. Isolated central nervous system (CNS) was used for electrophysiological experiments. Details of preparation and identification of neurons are given elsewhere (Ierusalimsky et al.1992; Balaban2002). Injection of cold isotonic $MgCl_2$ was made before the CNS isolation to minimize pain. Experimental procedures were in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and the protocol was approved by the Ethical Committee of the Institute of Higher Nervous Activity and Neurophysiology of Russian Academy of Sciences.

Electrophysiological experiments

Intracellular recordings from isolated brain ganglia were made using standard electrophysiological techniques. Identified withdrawal giant interneurons of the parietal ganglia (Pa3 and Pa2; Balaban2002) were penetrated with sharp glass microelectrodes filled with 2 M potassium acetate (tip resistance, 15–20 MOhm). The cutaneal and intestinal nerves were stimulated via plastic suction electrodes with 3 ms stimuli. The cutaneous and intestinal nerves were chosen because published data suggest that mechanosensory neurons receiving information from the skin (cutaneous nerve) form a glutamatergic-like synapses with the premotor neurons triggering withdrawal (Malyshev and Balaban2002;Bravarenko et al. 2003), while the neurons receiving information via intestinal nerve have quite different transmitter suggested to be acetylcholine (Ter-Markarian et al. 1990). Testing these two functionally and neurochemically different synaptic inputs to the same identified premotor neurons allowed us to widen the options for interpretation of results. Intensity of stimuli was adjusted in each experiment to elicit complexexcitatory postsynaptic potentials (EPSPs) of 5–12 mV amplitude from each stimulated nerve in the giant premotor interneurons. Intracellular signals were recorded with preamplifiers (Axoclamp 2B, Axon Instruments, CA, USA), digitized, and stored on a computer (Digidata 1400A A/D converter and Axoscope 10.0 software, both from Axon Instruments, CA, USA).

In the current study, we chose two distinct types of the training protocol: a strong training protocol (five tetanizations combined with five serotonin applications) to induce long-lasting LTP, and a weak training protocol based on pilot experiments (five tetanizations only or five tetanizations+single serotonin application) to induce a transient potentiation (Fig. 1). Both protocols were necessary for examination of the ability of HDAC inhibitors to affect the memory. The strong training protocol started with five test stimuli with a 10 min interstimulus interval, followed by tetanization (five high-frequency 10 Hz trains of stimuli, duration of each train 1 min, 5 min intervals between the trains, test stimulus amplitude was increased 10 fold), followed by posttetanic testing with a single stimuli of initial amplitude with 10 min interstimulus intervals for several hours. Serotonin was applied to the experimental bath just before each tetanization train to a final bath concentration of 10^{-5} M with washout in 2 min after each tetanization train. The weak training protocols started with five test stimuli with a 10 min interstimulus interval, followed by tetanization (five high-frequency 10 Hz trains of stimuli; duration of each train 1 min; with 5 min intervals between trains; test stimulus amplitude was increased 10 fold), followed by posttetanic testing with a single stimuli of initial amplitude with10 min interstimulus interval for several hours. Serotonin was applied to the experimental bath once before a tetanization train or not applied at all. The estimated final bath concentration of serotonin in all cases was 10^{-5} M. The EPSP amplitudes were scored for at least 4 h after the end of tetanizations. In our experiments, we investigated a homosynaptic plasticity, using the same nerve (cutaneal or intestinal) for test stimulation and tetanization.

Drugs

Methiothepin (MET) (Sigma, St. Louis, USA) – the established nonselective serotonergic receptors inhibitor, sodium butyrate (NaB) – a histone deacetylases inhibitor (Sigma, St. Louis, USA), and serotonin (5-HT) (Tocris, Bristol, UK) were dissolved in a sterile Ringer saline (in mM: 100 NaCl, 4 KCl, 7 $CaCl_2$, 5 $MgCl_2$, and 10 Tris-HCl buffer (pH 7.8)). Trichostatin A (TSA) – a histone deacetylases inhibitor (Sigma,

St. Louis, USA) was dissolved in DMSO to a concentration of 10 mM to make a stock solution. Estimated final bath concentration of methiothepin was $1.4x10^{-5}$M, sodium butyrate - $6x10^{-5}$M, trichostatin A – $0.5x10^{-6}$M, and serotonin - 10^{-5}M. These concentrations were shown to produce the significant effects of chosen chemical substances in pilot experiments in isolated nervous system of the snail. The specific timing at which the substances were applied is indicated in each figure.

Several experimental series were performed: (1) Recordings of the EPSP to test stimuli without any influences; (2) Tetanization accompanied by bath application of 5-HT in weak and strong training protocol models; (3) Tetanization accompanied by bath application of 5-HT with MET added to the bath 40 min before the tetanization in the strong training protocol model; (4) Tetanization accompanied by bath application of 5-HT with MET+NaB/ MET+TSA added to the bath 40 min before the tetanization in the strong training protocol model; (5) Tetanization accompanied by bath application of NaB/ TSA added to the bath 40 min before the tetanization in weak training protocol models.

In all experimental series the bath-perfused drugs (MET, NaB, TSA) were present for 40 min before the tetanization trains; the perfusion system was in the closed mode. After the last tetanization, the perfusion system was switched to an open state (washout), while the flow rate was 0.2 ml/min at a bath volume of 3 ml. In control experiments without tetanization and serotonin application, the perfusion system was switched to an open state at the same time.

Data analysis

All data are presented as the mean±S.E.M. Statistical analyses were performed using StatSoft Statistica version 10. Results were compared using ANOVA. $p < 0.05$ was predetermined as defining the statistically significant differences, and in figures was denoted by $*^{\#}$.

Results

In the first series of experiments, we investigated whether the inhibition of serotonergic receptors due to administration of MET would affect the LTP. As a first step, experiments without LTP induction were conducted to test whether prior application of serotonergic receptor inhibitor MET would affect synaptic transmission. In all control recordings, test stimulation without tetanization caused a gradual decrease in the complex EPSPs amplitudes in command neurons (habituation) (Control, n.cutaneus, n = 9, n.intestinalis, n = 9). In another series of experiments, MET was applied during the first 40 min of the experiment and was washed out after. The untetanized inputs (both nerves) (Fig. 2a, b) showed no significant changes in synaptic transmission under MET administration, except a few time points at cutaneal nerve stimulation (Control+MET, n.cutaneus, n = 8, n.intestinalis, n = 9) compared to the Control groups.

In the next series of experiments, we performed test stimulation of cutaneal nerve combined with its tetanization accompanied by the serotonin bath application. The baseline complex EPSPs were recorded for 40 minutes at 10-minute intervals. Five serotonin applications and tetanic stimulations elicited a

robust LTP that was constant over 240 minutes (Fig. 2c, group 5x(5-HT+tet), n = 10). Thus, 2 hours after the last tetanization and serotonin application, the EPSP amplitude was 138.1 ± 17.1% of the initial value, while in the Control group, the response attenuated, and at the same time point the EPSP amplitude was 63.1 ± 5.4%, ($p < 0.0001$). Four hours after tetanization, the amplitude of the EPSP of tetanized inputs also significantly exceeded the response amplitudes in the Control group (5x(5-HT+tet) group, 150.0 ± 23.6%; Control group, 31.6 ± 5.8%, $p < 0.0001$). Application of MET 40 minutes before tetanization + application of serotonin affected the amplitude of EPSP drastically: it caused markedly declined LTP (Fig. 2c, MET+5x(5-HT+tet) group, n = 10). So, 2 hours after the last tetanization + serotonin, the EPSP amplitude in the MET + 5x (5-HT + tet) group was 59.4 ± 13.1% and significantly differed from the EPSP amplitudes in the 5x (5-HT + tet) (138.1 ± 17.1%, $p < 0.005$). During the next 2 hours there was a gradual decrease in the amplitude of EPSP in MET+5x(5-HT+tet). So, 4 hours after the last tetanization + application of serotonin the EPSP amplitude in the MET + 5x (5-HT + tet) was 27.7 ± 4.7%, while in the 5x(5-HT+tet) group it was 150.0 ± 23.6% ($p < 0.0005$) (Fig. 3a).

In the next series of experiments, we performed test stimulation of intestinal nerve combined with its tetanization accompanied by the serotonin bath application. The baseline EPSP was recorded for 40 minutes at 10-minute intervals. Five serotonin application and tetanus stimulation elicited a robust LTP that was persistent over 240 minutes (Fig. 2d, group 5x(5-HT+tet), n = 11). Thus, 2 hours after the last tetanization and serotonin application, the EPSPs amplitude was 139.7 ± 14.4% of the initial value, while in the Control group, the response attenuated, and at the same time point the EPSP amplitude was 56.1 ± 12.1% ($p < 0.0005$). Four hours after tetanization, the amplitude of the EPSP of tetanized inputs also significantly exceeded the response amplitudes in the Control group (5x(5-HT+tet) group, 124.5 ± 9.8%; Control group, 36.8 ± 10.7%, $p < 0.0001$). MET application significantly impaired the EPSPs amplitude in MET+5x(5-HT+tet) group (n = 10) in comparison to the control (Fig. 2d) in experiments with stimulation of the intestinal nerve. For example, the levels of LTP, expressed as a percentage baseline and quantified at 2 hours after the tetanus, were 65.9 ± 16.0% in MET+5x(5-HT+tet) and 139.7 ± 14.4% in 5x(5-HT+tet) ($p < 0.005$). Remarkably, MET significantly influenced only the late phase of LTP. The EPSP amplitude in presence of MET was reduced to 27.9 ± 7.8% vs 124.5 ± 9.8% in 5x(5-HT+tet) at 4 hours after the tetanus ($p < 0.0001$) (Fig. 3b). It should be noted that MET application didn't change the EPSPs without the LTP induction. Thus, electrophysiological analysis revealed that repeated serotonin applications and tetanizations facilitated the EPSPs in the premotor interneurons, and this effect was suppressed by pharmacological inhibition of serotonergic receptors with MET.

In the second series of experiments, we investigated whether the increased histone acetylation upon administration of HDACis NaB or TSA would prevent the disruption of LTP by MET. First, we checked for possible effects of the HDACis on basal synaptic transmission. In all recordings, test stimulation without tetanization caused a gradual decrease in the EPSPs amplitudes in command neurons (habituation) (groups Control, n.cutaneus, n = 9, n.intestinalis, n = 9, Fig. 4a, b). In a separate series of experiments, NaB or TSA were applied during the first 40 min of the experiment and were washed out after. The untetanized inputs (both nerves) showed no significant changes in synaptic transmission under HDACis

administration (Fig. 4a, b, groups Control+NaB, n.cutaneus, n = 8, n.intestinalis, n = 8; Control+TSA, n.cutaneus, n = 9, n.intestinalis, n = 9) compared to the Control groups.

The next step was to find out whether the HDACis are able to rescue the MET-induced impairment of potentiation (Fig. 4c, d). In the next series of experiments, the cutaneal nerve was chosen for tetanization. The baseline EPSPs were recorded for 40 minutes at 10-minute intervals. Five serotonin applications and tetanic stimulations elicited the robust LTP that lasted over 240 minutes (Fig. 4c, group 5x(5-HT+tet), n = 11). NaB or TSA treatments combined with MET not only rescued the serotonin-evoked LTP, but increased it (Fig. 4c, NaB+MET+5x(5-HT+tet), n = 10, TSA+MET+5x(5-HT+tet) groups, n = 10). For example, the levels of LTP, quantified at 70 minutes after the first tetanus, were 191.7 ± 21.9% in NaB+MET+5x(5-HT+tet) and 196.4 ± 20.1% in TSA+MET+5x(5-HT+tet), these values significantly differed from 5x(5-HT+tet) (133.4 ± 10.7%) at this time point (NaB+MET+5x(5-HT+tet) vs. 5x(5-HT+tet), $p < 0.05$; TSA+MET+5x(5-HT+tet) vs. 5x(5-HT+tet), $p < 0.05$). Two hours after the last tetanization and serotonin application, the EPSPs amplitudes were 174.6 ± 23.9% and 177.8 ± 16.5% of the initial value in NaB+MET+5x(5-HT+tet) and TSA+MET+5x(5-HT+tet), respectively, in 5x(5-HT+tet) group the response at the same time point was 138.1 ± 17.1% ($p > 0.05$). Four hours after the tetanus, the EPSPs amplitudes were 143.2 ± 18.1% and 130.6 ± 13.8% of the initial value in NaB+MET+5x(5-HT+tet) and TSA+MET+5x(5-HT+tet), respectively, in 5x(5-HT+tet) group the response at the same time point was 150.0 ± 23.6% ($p > 0.05$) (Fig. 5a). We did not observe any difference between these groups during the late LTP.

In the next series of experiments, the intestinal nerve was chosen for tetanization. The baseline EPSPs were recorded for 40 minutes at 10-minute intervals. Five serotonin applications and tetanic stimulations elicited a robust LTP that was constant over 240 minutes (Fig. 4d, group 5x(5-HT+tet), n = 11). NaB or TSA treatment combined with MET significantly increased the serotonin-evoked LTP (NaB+MET+5x(5-HT+tet), n = 10, TSA+MET+5x(5-HT+tet) groups, n = 11). For example, the levels of LTP, quantified at 70 minutes after the first tetanus, were 190.6 ± 13.8% in NaB+MET+5x(5-HT+tet) and 198.2 ± 13.4% in TSA+MET+5x(5-HT+tet) which were significantly different from that of 5x(5-HT+tet) (149.8 ± 10.3%) at this time point (NaB+MET+5x(5-HT+tet) vs. 5x(5-HT+tet), $p < 0.05$; TSA+MET+5x(5-HT+tet) vs. 5x(5-HT+tet), $p < 0.05$). Two hours after the last tetanization and serotonin application, the EPSP amplitude was 173.4 ± 14.8% and 158.0 ± 7.6% of the initial value in NaB+MET+5x(5-HT+tet) and TSA+MET+5x(5-HT+tet) respectively, in 5x(5-HT+tet) group the response at the same time point was 139.7 ± 14.4% ($p > 0.05$). Four hours after the tetanus trains the EPSP amplitude was 118.8 ± 11.8% and 113.1 ± 10.2% of the initial value in NaB+MET+5x(5-HT+tet) and TSA+MET+5x(5-HT+tet) respectively, in 5x(5-HT+tet) group the response at the same time point was 124.5 ± 9.8% ($p > 0.05$) (Fig. 5b). We did not observe any difference between these groups during late LTP.

Thus, in experiments with a strong tetanization protocol, MET application led to a significant decrease in EPSP amplitude. When NaB or TSA was co-applied with MET, the EPSPs remained stably elevated. HDACis-mediated rescue effects were observed for both synaptic inputs (n.cutaneus, n.intestinalis).

These results suggest that HDAC blockade during LTP induction could rescue the impaired synaptic potentiation in the MET-treated isolated CNS via upregulation of the histone acetylation.

In an independent series of experiments, we tested whether the histone acetylation blockade can influence synaptic plasticity in experiments with a weak training protocol. There were no long-term effects in all experiments in which the weak training protocols were applied. In groups 5tet, 5tet+1x5-HT, NaB+5tet/TSA+5tet we observed a short-term increase of responses to cutaneal nerve stimulation in the first 90 minutes after the tetanization session (Figs. 6a, c) followed by a decrease in the EPSPs amplitudes below 100%. Similar results were observed for the intestinal nerve (Figs. 6b, d). At the same time, the amplitudes of the EPSPs in these groups during the entire time of the experiment practically did not differ from each other and were significantly less than 5x(5-HT+tet) at the late phase of potentiation. Altogether, the data obtained confirm that multiple 5-HT applications are necessary for LTP in this experimental model. However, applications of HDACis (NaB/ TSA) led to increase of both early and late LTP even in experiments with the weak training protocol with single serotonin application. For example, when the cutaneal nerve was tetanized, the levels of LTP, quantified at 60 minutes after the first tetanus, were 181.6 ± 18.6% in NaB+5tet+1x5-HT which were significantly different from that of 5x(5-HT+tet) (124.5 ± 11.5%) at this time point ($p < 0.05$). The EPSP amplitude at 2 hours after the last tetanization and serotonin application in the presence of NaB/ TSA was 148.9 ± 16.3% (NaB+5tet+1x5-HT, $n = 10$) and 134.6 ± 14.3% (TSA+5tet+1x5-HT, $n = 10$) what was comparable to 5x(5-HT+tet) (138.1 ± 17.1%, $n = 10$, $p > 0.05$). Late phase of LTP (4 hours after tetanus) in the presence of NaB/ TSA was comparable to 5x(5-HT+tet) (NaB+5tet+1x5-HT, 149.5 ± 20.3%, TSA+5tet+1x5-HT, 107.0 ± 15.2%, 5x(5-HT+tet), 150.5 ± 23.6%, $p > 0.05$) (Fig. 7a). When the intestinal nerve was tetanized, the levels of LTP, quantified at 60 minutes after the first tetanus, were 240.3 ± 20.3% in NaB+5tet+1x5-HT, which were significantly different from that of 5x(5-HT+tet) (147.3 ± 13.1%) at this time point ($p < 0.001$). In the presence of TSA, the levels of LTP, quantified at 60 minutes after the first tetanus, were 205.2 ± 16.7% in TSA+5tet+1x5-HT, which were significantly different from that of 5x(5-HT+tet) (147.3 ± 13.1%) at this time point ($p < 0.005$). The EPSP amplitude at 2 hours after the last tetanization and serotonin application in the presence of NaB/ TSA was 140.1 ± 23.7% (NaB+5tet+1x5-HT, $n = 10$) and 181.0 ± 29.3% (TSA+5tet+1x5-HT, $n = 10$), what was comparable to 5x(5-HT+tet) (139.7 ± 14.4%, $n = 10$, $p > 0.05$). Late phase of LTP (4 hours after tetanus trains) in the presence of NaB/ TSA was comparable to 5x(5-HT+tet) (NaB+5tet+1x5-HT, 95.9 ± 15.4%, TSA+5tet+1x5-HT, 125.9 ± 17.6%,5x(5-HT+tet), 124.5 ± 9.8%, $p > 0.05$) (Fig. 7b). Thus, the EPSPs amplitudes in weak protocol experiments showed the following trend: potentiation was observed during early phase of LTP but the late phase at the end of experiments was absent. It was curious that application of NaB or TSA without serotonin produced potentiation lasting only minutes that gradually declined to the pre-tetanic values. Only in the presence of single pulse of 5-HT and NaB or TSA, the LTP was induced and lasted over 240 minutes which was comparable to the potentiation induced by five pulses of 5-HT.

Discussion

First of all, it should be noted that LTP in mollusks provides a valuable model for studying the molecular mechanisms underlying synaptic plasticity. In all series of our electrophysiological experiments, a session of five tetanizing stimuli associated with 5-HT applications led to a significant long-term increase of neuronal synaptic responses. All experiments without tetanization showed incremental decrease in responses during 5 h of experiment that is consistent with the literature data obtained on this model (Malyshev and Balaban 2002) (Figs. 2, 4, 6). Responses to stimulation of cutaneous and intestinal nerves changed similarly in spite of their different innervation targets and presumed different transmitters of presynaptic sensory neurons involved (Ter-Markarian et al. 1990; Bravarenko et al. 2003).

In the present study we examined whether the nonselective serotonin receptors inhibitor MET shown to effectively impair the learning in behavioral experiments in *Helix* (Abramova et al. 2006; Solntseva and Nikitin 2008) would impair the long-term plasticity in isolated CNS of *Helix*. We found that a strong training protocol that typically induced LTP, induced only a transient potentiation in the presence of MET (Fig. 2c, d). When the cutaneous nerve was used for stimulation, application of MET induced a potent inhibition of potentiation beginning almost immediately after the last presentation of tetanization+serotonin. No differences were observed only during the first 40 min after beginning of tetanization. When the intestinal nerve was used for stimulation, the observations were the same – in the presence of MET a potent inhibition of potentiation started almost immediately after the last presentation of tetanization+serotonin. No differences were observed only during the first 40 minutes after the last tetanization+serotonin. Thus, MET alone dramatically decreased both the early and the late phases of LTP showing important role of serotonergic receptors/ serotonin and their cascades in changes of synaptic plasticity occurring after the LTP induction. These data are consistent with earlier works, where it was shown that methiothepin disrupts the acquisition of both sensitization of defensive reaction in snails (Abramova et al. 2006) and consolidation of associative reflexes (Solntseva and Nikitin 2008).

Next, we tested whether the HDAC inhibition could rescue the LTP deficits in the MET-treated isolated CNS of *Helix*. When isolated CNS was perfused simultaneously with a blocker of serotonin receptors MET and HDACis (NaB or TSA) before LTP induction, a long-lasting potentiation was observed. HDACis NaB or TSA prevented the weakening of potentiation, in other words, they rescued the synaptic plasticity deficit. These results are fully consistent with our previous study in which simultaneous administration of histone deacetylase inhibitor sodium butyrate and a blocker of serotonin receptors methiothepin under conditions of memory reactivation prevented weakening of the context memory in mollusks (Zuzina et al. 2021). Similar ability of HDACis to act as a cognitive enhancers was demonstrated in a number of earlier works (Alarco´n et al. 2004; Korzus et al. 2004; Barichello et al. 2015; Barichello et al. 2015; Ko et al. 2016; Shivakumar et al. 2020; Zuzina et al. 2020; Vinarskaya et al. 2021).

In a separate series of experiments, we examined how the HDAC inhibition affects synaptic plasticity induced by the weak training protocols. The protocol of weak memory was shown to be informative in certain conditions in *Aplysia* (Guan et al. 2002; Chen et al. 2014; Pearce et al. 2017). Less that 5 bouts of tail shocks and less that 5 serotonin applications resulted in a weak memory in *Aplysia* experiments. In our experiments were used 5 tetanizing stimuli with 5 serotonin applications for triggering development

of a full-scale LTP of synaptic responses, and 5 tetanizing stimuli with no or single serotonin application for formation of a weak memory. We demonstrated that the weak training protocols (five tetanizations or five tetanizations+single serotonin application) yielded in only a transient short-term potentiation (Fig. 6). HDACis application practically did not influence synaptic plasticity in experiments with weak training protocol without serotonin – only a transient potentiation of responses was observed, no difference from the control series. One pulse of 5-HT combined with five tetatus trains which normally induced only the short-term synaptic plasticity, in the presence of HDACis induced LTP: transient early LTP transformed into a long-lasting LTP. Experiments with TSA conducted in *Aplysia* are consistent with this data. In the presence of TSA, one pulse of 5-HT, which normally induced only a short-term synaptic plasticity, induced LTP (Guan et al. 2002; Chen et al. 2014). Interestingly, in our experiments the HDACis were shown to affect the EPSPs amplitudes during early phase of LTP in NaB+5tet+1x5-HT and TSA+5tet+1x5-HT groups. The EPSPs amplitudes in these groups significantly increased under the NaB or TSA administration during the first hour after the induction of LTP in comparison with the control LTP in 5x(5-HT+tet) group. However, later the EPSPs amplitudes in NaB+5tet+1x5-HT and TSA+5tet+1x5-HT groups gradually returned to basal potentiated levels of 5x(5-HT+tet) group during the late phase (Fig. 6). Kolotova et al. (2021) also reported that histone deacetylase inhibitor, sodium butyrate, could act as an enhancer of synaptic potentiation in premotor interneurons, increasing the amplitude of LTP. Thus, we demonstrated that tetanus+single serotonin application in combination with HDAC blockade could mimic the tetanus+repeated serotonin applications and be enough for the sustained, long-lasting synaptic changes, similar to the changes reported in the literature (Silva et al. 1998; Kandel 2001; Dudai 2004; Kelleher et al. 2004; Klann and Dever 2004; Sutton and Schuman 2006).

Thus, in the present study we showed that histone acetylation status in the process of plasticity development contributes to the increase in synaptic efficiency. We can only speculate about molecular mechanisms through which the observed enhancement of plasticity occurs. Given that serotonin can modify the histone acetylation status in the context of learning-related serotonin-dependent synaptic plasticity (Guan et al. 2002) and our findings, we may assume that in our model serotonin also may affect the histone acetylation status. We used applications of two different HDAC inhibitors, sodium butyrate or trichostatin A, both known to result in histone hyperacetylation (Marks et al. 2004; Marks and Dokmanovic 2005), and a subsequent potentiation of memory-related gene expression (Brownell and Allis 1996; Levenson and Sweatt 2005; Vecsey et al. 2007; Gräff et al. 2014). Presence of NaB and TSA in our experiments caused the same effect on the LTP amplitude as an additional four serotonin applications, and stabilization of transient potentiation into a long-lasting in experiments with one pulse of 5-HT+tetanus. We speculate that after a single pulse of serotonin+tetanus there are some changes in acetylation of histones compared to the basal levels, nevertheless, these changes are not sufficient, and we observe only a short-term synaptic plasticity. The sufficient level of histone acetylation for LTP is achieved either by the five pulses of 5-HT+tetanus or a combination of single serotonin pulse+tetanus with HDACis.

Below, we try to describe a molecular pathway through which the serotonin signaling may target the histone acetylation. Using calcium sensors it was directly shown that serotonin application or

intracellular stimulation of a single identified serotonergic neuron caused a lasting increase in intracellular calcium concentration in identified premotor interneurons used in the present experiments (Balaban et al. 2004). It is well known that in the absence of free calcium, nitric oxide (NO) synthase (NOS) is inactive even with excessive concentrations of substrate, amino acid l-arginine (Andrew and Mayer 1999), and an increase of intracellular calcium leads to a rapid catalytic activation of NOS (Förstermann and Sessa 2012) and additional NO synthesis. A range of data showed that NO affects synaptic plasticity in several species of gastropod mollusks both in vivo (Robertson et al. 1994; Elphick et al. 1995; Hawkins 1996; Teyke 1996; Katzoff et al. 2002; Sakura et al. 2004; Yabumoto et al. 2008; Balaban et al. 2011) and in vitro (Jacklet 1997; Malyshev and Balaban 1999; Kemenes et al. 2002; Antonov et al. 2007). In the terrestrial snail *Helix,* it was shown that NO is necessary for LTP of synaptic responses and for development of the context memory (Korshunova and Balaban 2014). The NO-synthase inhibitor L-NNA was shown to block postsynaptically induced potentiation of synaptic inputs in withdrawal interneurons of *Helix* (Malyshev and Balaban 1999). Moreover, NO complements the serotonin action in *Helix* neurons (Diakonova 2000; Inoue et al. 2001). At present, nitrosylation of amino acid residues (predominantly, cysteine) in the target proteins is described as one of the main signaling mechanisms for NO (Bradley and Steinert 2016). Recent studies suggest a role for NO in epigenetic mechanisms related to the acetylation of histone protein. HDACs were shown to be the potential target proteins in the cell for direct nitrosylation. It was demonstrated that NO-dependent S-nitrosylation of HDAC2 led to dissociation of the enzyme from the CREB-regulated gene promoters, thereby increasing acetylation of histones at specific promoter regions and transcription of several genes, including c-fos, erg1, VGP and nNOS (Nott et al. 2008; Nott and Riccio 2009). Wattson and Riccio (2009) got similar data – the NO donor S-nitrosoglutathione inhibited the HDAC2 activity in embryonic cortical neurons, and caused an increase in histone acetylation. It has recently been shown that HDAC6 (Okuda et al. 2015) as well as HDAC2 (Riccio et al. 2006; Nott et al. 2008; Resstel et al. 2008; Gräff et al. 2014) are targets for the NO. Gräff et al. (2014) managed to demonstrate that serotonin leads to histone hyperacetylation and the expression of neuroplasticity-related genes by stimulating HDAC2 nitrosylation and its subsequent dissociation from the chromatin. Collectively, these data provide a mechanism for serotonin-mediated synaptic potentiation through the increased histone acetylation. It is worth to note that we realize that serotonin may target diverse cellular substrates and that histone acetylation may be only one of them. Although we still do not fully understand the molecular mechanism by which serotonin and histone acetylation are connected, there is good reason to believe that this link exists and that enhanced acetylation level (thereby, upregulated transcription) is part of the molecular mechanism for turning on the genes' expression during LTP in terrestrial snail.

Abbreviations

CNS Central nervous system

HDAC Histone deacetylase

HDACi Histone deacetylase inhibitor

LTP Long-term potentiation

MET Methiothepin

NaB Sodium butyrate

NO Nitric oxide

NOS Nitric oxide synthase

TSA Thichostatin A

Declarations

Aknowledgments. This study was supported by grant of Russian Ministry of Education and Science № 075–15–2020-801.

Competing Interests. The authors declare that they have no conflict of interest.

Author Contributions. The authors contributed equally. All authors read and approved the final manuscript.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving snails were in accordance with the ethical standards and approved (#012 from 10.10.2014) by Ethical Committee of the Institute of Higher Nervous Activity and Neurophysiology of Russian Academy of Sciences.

References

1. Abramova MS, Nistratova VL, Moskvitin AA, Pivovarov AS (2006) Methiothepin-sensitive serotonin receptors are involved in the postsynaptic mechanism of sensitization of the defensive response in the common snail. Neurosci Behav Physiol 36:589–596. https://doi.org/10.1007/s11055-006-0062-4
2. Agudelo M, Yoo C, Nair MP (2012) Alcohol-induced serotonergic modulation: the role of histone deacetylases. Alcohol 46:635–642. https://doi.org/10.1016/j.alcohol.2012.03.005
3. Alarcon JM, Malleret G, Touzani K, Vronskaya S, Ishii S, Kandel ER, Barco A (2004) Chromatin acetylation, memory, and LTP are impaired in CBP+/− mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron 42:947–959. https://doi.org/10.1016/j.neuron.2004.05.021
4. Alberini CM, Kandel ER (2014) The regulation of transcription in memory consolidation. Cold Spring Harb Perspect Biol 7:a021741. https://doi.org/10.1101/cshperspect.a021741
5. Andrew PJ, Mayer B (1999) Enzymatic function of nitric oxide synthases. Cardiovasc Res 43:521–531. https://doi.org/10.1016/s0008-6363(99)00115-7

6. Antonov I, Ha T, Antonova I, Moroz LL, Hawkins RD (2007) Role of nitric oxide in classical conditioning of siphon withdrawal in Aplysia. J Neurosci 27:10993–11002. https://doi.org/10.1523/JNEUROSCI.2357-07.2007
7. Asaoka N, Nagayasu K, Nishitani N, Yamashiro M, Shirakawa H, Nakagawa T, Kaneko S (2015) Inhibition of histone deacetylases enhances the function of serotoninergic neurons in organotypic raphe slice cultures. Neurosci Lett 593:72–77. https://doi.org/10.1016/j.neulet.2015.03.028
8. Balaban PM (2002) Cellular mechanisms of behavioral plasticity in terrestrial snail. Neurosci Biobehav Rev 26:597–630. https://doi.org/10.1016/s0149-7634(02)00022-2
9. Balaban P, Bravarenko N (1993) Long-Term sensitization and environmental conditioning in terrestrial snails. Exp Brain Res 96:487–493. https://doi.org/10.1007/BF00234116
10. Balaban PM, Vehovszky A, Maksimova OA, Zakharov IS (1987) Effect of 5,7-dihydroxytryptamine on the food-aversive conditioning in the snail Helix lucorum L. Brain Research 404:201–210. https://doi.org/
11. Balaban PM, Korshunova TA, Bravarenko NI (2004) Postsynaptic calcium contributes to reinforcement in a three-neuron network exhibiting associative plasticity. Eur J Neurosci 19:227–233. https://doi.org/10.1016/0006-8993(87)91371-0
12. Balaban PM, Roshchin MV, Korshunova TA (2011) Two-faced nitric oxide is necessary for both erasure and consolidation of memory. Zh Vyssh Nerv Deiat Im IP Pavlova 61:274–280.
13. Balaban PM, Vinarskaya AK, Zuzina AB, Ierusalimsky VN, Malyshev AY (2016) Impairment of the serotonergic neurons underlying reinforcement elicits extinction of the repeatedly reactivated context memory. Sci Rep 6:36933. https://doi.org/10.1038/srep36933
14. Barichello T, Generoso JS, Simões LR et al (2015) Sodium butyrate prevents memory impairment by re-establishing BDNF and GDNF expression in experimental pneumococcal meningitis. Mol Neurobiol 52:734–740. https://doi.org/10.1007/s12035-014-8914-3
15. Bence M, Koller J, Sasvari-Szekely M, Keszler G (2012) Transcriptional modulation of monoaminergic neurotransmission genes by the histone deacetylase inhibitor trichostatin A in neuroblastoma cells. J Neural Transm (Vienna) 119:17–24. https://doi.org/10.1007/s00702-011-0688-4
16. Bradley SA, Steinert JR (2016) Nitric Oxide-Mediated Posttranslational Modifications: Impacts at the Synapse. Oxid Med Cell Longev 2016:5681036. https://doi.org/10.1155/2016/5681036
17. Bravarenko NI, Korshunova TA, Malyshev AY, Balaban PM (2003) Synaptic contact between mechanosensory neuron and withdrawal interneuron in terrestrial snail is mediated by l-glutamate-like transmitter. Neurosci Let 341:237–240. https://doi.org/10.1016/s0304-3940(03)00213-1
18. Brownell JE, Allis CD (1996) Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr Opin Genet Dev 6:176–184. https://doi.org/10.1016/s0959-437x(96)80048-7
19. Campbell RR, Wood MA (2019) How the epigenome integrates information and reshapes the synapse. Nat Rev Neurosci 20:133–147. https://doi.org/10.1038/s41583-019-0121-9

20. Chen S, Cai D, Pearce K, Sun PY, Roberts AC, Glanzman DL (2014) Reinstatement of long-term memory following erasure of its behavioral and synaptic expression in Aplysia. ELife 3:e03896. https://doi.org/
21. Deryabina IB, Muranova LN, Andrianov VV, Gainutdinov KL (2018) Impairing of serotonin synthesis by p-chlorphenylanine prevents the forgetting of contextual memory after reminder and the protein synthesis inhibition. Front Pharmacol 9:607. https://doi.org/10.7554/eLife.03896
22. Diakonova TL (2000) Interaction of serotonin and nitric oxide (NO) in activation of the serotoninergic system in Helix. Ross Fiziol Zh Im IM Sechenova 86:1210–1219.
23. Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? Annu. Rev. Psychol 55:51–86. https://doi.org/10.1146/annurev.psych.55.090902.142050
24. Elphick MR, Kemenes G, Staras K, O'Shea M (1995) Behavioral role for nitric oxide in chemosensory activation of feeding in a mollusc. J Neurosci 15:7653–7664. https://doi.org/10.1523/JNEUROSCI.15-11-07653.1995
25. Federman N, Fustiñana MS, Romano A (2009) Histone acetylation is recruited in consolidation as a molecular feature of stronger memories. Learn Mem 16:600–606. https://doi.org/10.1101/lm.1537009
26. Federman N, Fustiñana MS, Romano A (2012) Reconsolidation involves histone acetylation depending on the strength of the memory. Neurosci 219:145–156. https://doi.org/
27. Förstermann U, Sessa WC (2012) Nitric oxide synthases: regulation and function. Eur Heart J 33:829–837. https://doi.org/10.1016/j.neuroscience.2012.05.057
28. Gräff J, Joseph NF, Horn ME et al (2014) Epigenetic priming of memory updating during reconsolidation to attenuate remote fear memories. Cell 156:261–276. https://doi.org/10.1016/j.cell.2013.12.020
29. Guan Z, Giustetto M, Lomvardas S et al (2002) Integration of Long-Term-Memory-Related Synaptic Plasticity Involves Bidirectional Regulation of Gene Expression and Chromatin Structure. Cell 111:483–493. https://doi.org/10.1016/s0092-8674(02)01074-7
30. Hawkins RD (1996) NO honey, I don't remember. Neuron 16:465–467. https://doi.org/10.1016/s0896-6273(00)80064-1
31. Holloway T, González-Maeso J (2015) Epigenetic Mechanisms of Serotonin Signaling. ACS Chem Neurosci 6:1099–1109. https://doi.org/10.1021/acschemneuro.5b00033
32. Hu YT, Tang CK, Wu CP, Wu PC, Yang EC, Tai CC, Wu YL (2018) Histone deacetylase inhibitor treatment restores memory-related gene expression and learning ability in neonicotinoid-treated Apis Mellifera. Insect Mol Bio 27:512–521. https://doi.org/10.1111/imb.12390
33. Ierusalimsky VN, Zakharov IS, Palikhova TA, Balaban PM (1992) The nervous system and the mapping of the neurons of Gastopoda mollusk Helix lucorum L. Zh Vyssh Nerv Deiat Im IP Pavlova 42:1075–1089.
34. Inoue T, Watanabe S, Kirino Y (2001) Serotonin and NO complementarily regulate generation of oscillatory activity in the olfactory CNS of a terrestrial mollusk. J Neurophysiol 85:2634–2638.

https://doi.org/10.1152/jn.2001.85.6.2634
35. Jacklet JW (1997) Nitric oxide signaling in invertebrates. Invert Neurosci 3:1–14. https://doi.org/10.1007/BF02481710
36. Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038. https://doi.org/10.1126/science.1067020
37. Kandel ER, Schwartz JH (1982) Molecular biology of an elementary form of learning: modulation of transmitter release by cyclic AMP. Science 218:433–443. https://doi.org/10.1126/science.6289442
38. Katzoff A, Ben-Gedalya T, Susswein AJ (2002) Nitric oxide is necessary for multiple memory processes after learning that a food is inedible in Aplysia. J Neurosci 22:9581–9594. https://doi.org/10.1523/JNEUROSCI.22-21-09581.2002
39. Kemenes I, Kemenes G, Andrew RJ, Benjamin PR, O'Shea M (2002) Critical time-window for NO-cGMP-dependent longterm memory formation after one-trial appetitive conditioning. J Neurosci 22:1414–1425. https://doi.org/10.1523/JNEUROSCI.22-04-01414.2002
40. Ko HG, Kim JI, Sim SE et al (2016) The role of nuclear PKMζ in memory maintenance. Neurobiol Learn Mem 135:50–56. https://doi.org/10.1016/j.nlm.2016.06.010
41. Korshunova TA, Balaban PM (2014) Nitric oxide is necessary for long-term facilitation of synaptic responses and for development of context memory in terrestrial snails. Neurosci 266:127–135. https://doi.org/10.1016/j.neuroscience.2014.02.004
42. Kelleher RJ 3rd, Govindarajan A, Tonegawa S (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 44:59–73. https://doi.org/10.1016/j.neuron.2004.09.013
43. Klann E, Dever TE (2004) Biochemical mechanisms for translational regulation in synaptic plasticity. Nat Rev Neurosci 5:931–942. https://doi.org/10.1038/nrn1557
44. Kolotova DE, Malyshev AYu, Balaban PM (2021) Histone deacytylase inhibitor enhances long-term synaptic potentiation in neurons of a grape snail. J Evolut Biochem Phys 57:704–708. https://doi.org/10.1134/s0022093021030170
45. Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42:961–972. https://doi.org/10.1016/j.neuron.2004.06.002
46. Lattal KM, Barrett RM, Wood MA (2007) Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. Behav Neurosci 121:1125–1131. https://doi.org/10.1037/0735-7044.121.5.1125
47. Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD (2004) Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 279:40545–40559. https://doi.org/10.1074/jbc.M402229200
48. Levenson JM, Sweatt JD (2005) Epigenetic mechanisms in memory formation. Nat Rev Neurosci 6:108–118. https://doi.org/10.1038/nrn1604

49. Malyshev AY, Balaban PM (1999) Synaptic facilitation in Helix neurons depends upon postsynaptic calcium and nitric oxide. Neurosci Lett 261:65–68. https://doi.org/10.1016/s0304-3940(98)01010-6
50. Malyshev AY, Balaban PM (2002) Identification of mechanoafferent neurons in terrestrial snail: response properties and synaptic connections. J Neurophysiol 87:2364–2371. https://doi.org/10.1152/jn.00185.2001
51. Marks PA, Richon VM, Miller T, Kelly WK (2004) Histone deacetylase inhibitors. Adv Cancer Res 91:137–168. https://doi.org/10.1016/S0065-230X(04)91004-4
52. Marks PA, Dokmanovic M (2005) Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs 14:1497–1511. https://doi.org/10.1517/13543784.14.12.1497
53. Nott A, Watson PM, Robinson JD, Crepaldi L, Riccio A (2008) S-Nitrosylation of histone deacetylase 2 induces chromatin remodelling in neurons. Nature 455:411–415. https://doi.org/10.1038/nature07238
54. Nott A, Riccio A (2009) Nitric oxide-mediated epigenetic mechanisms in developing neurons. Cell Cycle 8:725–730. https://doi.org/10.4161/cc.8.5.7805
55. Okuda K, Ito A, Uehara T (2015) Regulation of Histone Deacetylase 6 Activity via S-Nitrosylation. Biol Pharm Bull 38:1434–1437. https://doi.org/10.1248/bpb.b15-00364
56. Pearce K, Cai D, Roberts AC, Glanzman DL (2017) Role of protein synthesis and DNA methylation in the consolidation and maintenance of long-term memory in Aplysia. Elife 6:e18299. https://doi.org/10.7554/eLife.18299
57. Peixoto L, Abel T (2013) The role of histone acetylation in memory formation and cognitive impairments. Neuropsychopharmacol 38:62–76. https://doi.org/10.1038/npp.2012.86
58. Penney J, Tsai LH (2014) Histone deacetylases in memory and cognition. Sci Signal 7:re12. https://doi.org/10.1126/scisignal.aaa0069
59. Resstel LB, Corrêa FM, Guimarães FS (2008) The expression of contextual fear conditioning involves activation of an NMDA receptor-nitric oxide pathway in the medial prefrontal cortex. Cereb Cortex 18:2027–2035. https://doi.org/10.1093/cercor/bhm232
60. Riccio A, Alvania RS, Lonze BE et al (2006) A nitric oxide signaling pathway controls CREB-mediated gene expression in neurons. Molecular Cell 21:283–294. https://doi.org/10.1016/j.molcel.2005.12.006
61. Robertson JD, Bonaventura J, Kohm AP (1994) Nitric oxide is required for tactile learning in Octopus vulgaris. Proc R Soc Lond B Biol Sci 256:269–273. https://doi.org/10.1098/rspb.1994.0080
62. Sakura M, Kabetani M, Watanabe S, Kirino Y (2004) Impairment of olfactory discrimination by blockade of nitric oxide activity in the terrestrial slug Limax valentianus. Neurosci Lett 370:257–261. https://doi.org/10.1016/j.neulet.2004.08.025
63. Shivakumar M, Subbanna S, Joshi V, Basavarajappa BS (2020) Postnatal ethanol exposure activates HDAC-mediated histone deacetylation, impairs synaptic plasticity gene expression and behavior in mice. Int J Neuropsychopharmacol 23:324–338. https://doi.org/10.1093/ijnp/pyaa017

64. Silva AJ, Kogan JH, Frankland PW, Kida S (1998) CREB and memory. Annu Rev Neurosci 21:127–148. https://doi.org/10.1146/annurev.neuro.21.1.127
65. Solntseva SV, Nikitin VP (2008) Neurochemical mechanisms of food aversion conditioning consolidation in snail Helix lucorum. Ross Fiziol Zh Im IM Sechenova 94:1259–1269.
66. Stefanko DP, Barrett RM, Ly AR, Reolon GK, Wood MA (2009) Modulation of long-term memory for object recognition via HDAC inhibition. Proc Natl Acad Sci USA 106:9447–9452. https://doi.org/10.1073/pnas.0903964106
67. Sutton MA, Schuman EM (2006) Dendritic protein synthesis, synaptic plasticity, and memory. Cell 127:49–58. https://doi.org/10.1016/j.cell.2006.09.014
68. Ter-Markarian AG, Palikhova TA, Sokolov EN (1999) The action of atropine and d-tubocurarine on the monosynaptic connections between identified neurons in the central nervous system of the edible snail. Zh Vyssh Nerv Deiat Im IP Pavlova 40:183–184.
69. Teyke T (1996) Nitric oxide, but not serotonin, is involved in acquisition of food-attraction conditioning in the snail Helix pomatia. Neurosci Lett 206:29–32. https://doi.org/10.1016/0304-3940(96)12434-4
70. Vecsey CG, Hawk JD, Lattal KM et al (2007) Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J Neurosci 27:6128–6140. https://doi.org/10.1523/JNEUROSCI.0296-07.2007
71. Villain H, Florian C, Roullet P (2016) HDAC inhibition promotes both initial consolidation and reconsolidation of spatial memory in mice. Sci Rep 6:27015. https://doi.org/10.1038/srep27015
72. Vinarskaya AK, Balaban PM, Roshchin MV, Zuzina AB (2021) Sodium butyrate as a selective cognitive enhancer for weak or impaired memory. Neurobiol Learn Mem 180:107414. https://doi.org/10.1016/j.nlm.2021.107414
73. Watson PM, Riccio A (2009) Nitric oxide and histone deacetylases: A new relationship between old molecules. Commun Integr Biol 2:11–13. https://doi.org/10.4161/cib.2.1.7301
74. Yabumoto T, Takanashi F, Kirino Y, Watanabe S (2008) Nitric oxide is involved in appetitive but not aversive olfactory learning in the land mollusk Limax valentianus. Learn Mem 15:229–232. https://doi.org/10.1101/lm.936508
75. Zhu LJ, Sun YQ, Wang S, Shi HJ, Li N (2021) Involvement of 5-HT1A receptor-mediated histone acetylation in the regulation of depression. Neuroreport 32:1049–1057. https://doi.org/10.1097/WNR.0000000000001693
76. Zuzina AB, Vinarskaya AK, Balaban PM (2019) Increase in serotonin precursor levels reinstates the context memory during reconsolidation. Invert Neurosci 19:8. https://doi.org/10.1007/s10158-019-0227-9
77. Zuzina AB, Vinarskaya AKh, Balaban PM (2020) Histone deacetylase inhibitors rescue the impaired memory in terrestrial snails. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 206:639–649. https://doi.org/10.1007/s00359-020-01422-w

78. Zuzina AB, Vinarskaya AKh, Balaban PM, Roshchin MV (2021) Histone Deacetylase Inhibitor Prevents Memory Impairment by Methiothepin. In: Advances in cognitive research, artificial intelligence and neuroinformatics. Springer, Frankfurt. pp 619–631

Figures

Figure 1

Schematic representations of protocols. a – Strong training protocol (five tetanizations combined with five serotonin applications). b – Weak training protocol (five tetanizations+single serotonin application). c – Weak training protocol (five tetanizations only)

Figure 2

Effects of the serotonergic receptors blocker MET on synaptic plasticity. a, b – the untetanized inputs (Control, n.cutaneus, n = 9, n.intestinalis, n = 9) showed no significant changes in synaptic transmission under MET administration (Control+MET, n.cutaneus, n = 8, n.intestinalis, n = 9). c, d – MET blocked the induction of long-lasting LTP: MET application significantly impaired the increase in the EPSPs amplitudes in MET+5x(5-HT+tet) groups (n.cutaneus, n = 10, n.intestinalis, n = 10) in comparison to the control 5x(5-HT+tet) groups (n.cutaneus, n = 10, n.intestinalis, n = 11). The duration of drug infusion is shown as a rectangle at the bottom. The arrows (0 min at the scale) mark the the timing of the tetanic stimulations and serotonin applications. All data are presented as mean ± SEM. * denotes $p < 0.05$ MET+5x(5-HT+tet) vs. 5x(5-HT+tet); $^{\#}$ denotes $p < 0.05$ Control+MET vs. Control

Figure 3

Examples of complex EPSPs in withdrawal interneurons (LPa2, LPa3) evoked by stimulation of cutaneous (a) or intestinal (b) nerves. 1 – group 5x(5-HT+tet). 2 – group MET+5x(5-HT+tet). For every neuron the responses at time points 40 min (left panel I), 120 min after the last tetanic stimulation (middle panel II), and 230 min after the last tetanic stimulation (right panel III) are shown. Scale bars=5 mV, 500 ms

Figure 4

Histone acetylation inhibitors regulate the long-term plasticity. a, b – the untetanized inputs (Control, n.cutaneus, n = 9, n.intestinalis, n = 9) showed no significant changes in synaptic transmission under NaB or TSA administration (Control+NaB, n.cutaneus, n = 8, n.intestinalis, n = 8; Control+TSA, n.cutaneus, n = 9, n.intestinalis, n = 9). c, d – simultaneous administration of NaB or TSA and a blocker of serotonin receptors MET before LTP initiation prevents weakening of the potentiation in mollusk. NaB or TSA led to potentiation of the EPSPs amplitudes during the early phase of potentiation in NaB+MET+5x(5-HT+tet) groups (n.cutaneus, n = 10, n.intestinalis, n = 10)/ TSA+MET+5x(5-HT+tet) groups (n.cutaneus, n = 10, n.intestinalis, n = 11) in comparison to control 5x(5-HT+tet) groups (n.cutaneus, n = 10, n.intestinalis, n = 11), while there was no differences at time points corresponding to the late phase of potentiation. The duration of drugs infusion is shown as a rectangle at the bottom. The arrows (0 min at the scale) mark the timing of the tetanic stimulations and serotonin applications. All data are presented as mean ± SEM. * denotes $p < 0.05$ NaB+MET+5x(5-HT+tet) vs. 5x(5-HT+tet), $^{\#}$ denotes $p < 0.05$ TSA+MET+5x(5-HT+tet) vs. 5x(5-HT+tet)

Figure 5

Examples of complex EPSPs in withdrawal interneurons evoked by stimulation of cutaneous (a) or intestinal (b) nerves. 1 – group 5x(5-HT+tet). 2 – group NaB+MET+5x(5-HT+tet). 3 – group TSA+MET+5x(5-HT+tet). For every neuron the responses at time points -40 min (left panel I), 70 minutes after the first tetanus (middle panel II), 120 min after the last tetanic stimulation (middle panel III), and 230 min after the last tetanic stimulation (right panel IV) are shown. Scale bars=5 mV, 500 ms

Figure 6

HDAC inhibition affects the potentiation induced by the weak training protocols. Weak training protocols (five tetanizations only or five tetanizations+single serotonin application) induced a transient early potentiation (5tet, n.cutaneus, n = 10, n.intestinalis, n = 11; 5tet+1x5-HT, n.cutaneus, n = 10, n.intestinalis, n = 12) that significantly decreased at the time points corresponding to the late phase of LTP if compared to 5x(5-HT+tet) groups (n.cutaneus, n = 10, n.intestinalis, n = 11). Preincubation for 40 min with NaB/ TSA paired with five tetanizations and one pulse of 5-HT (NaB+5tet+1x5-HT, n.cutaneus, n = 10, n.intestinalis, n = 10; TSA+5tet+1x5-HT, n.cutaneus, n = 10, n.intestinalis, n = 10) induced the LTP comparable to that induced by five pulses of 5-HT+five tetanizations. However, exposure to NaB/ TSA paired to five tetanizations without serotonin had no long-term effect on potentiation (NaB+5tet, n.cutaneus, n = 10, n.intestinalis, n = 10; TSA+5tet, n.cutaneus, n = 9, n.intestinalis, n = 10). The duration of drugs infusion is shown as a rectangle at the bottom. The arrows (0 min at the scale) mark the timing of the tetanus and serotonin application. All data are presented as mean ± SEM. * denotes $p < 0.05$ NaB+5tet+1x5-HT or TSA+5tet+1x5-HT vs. 5x(5-HT+tet)

Figure 7

Examples of complex EPSPs in withdrawal interneurons evoked by stimulation of cutaneous (a) or intestinal (b) nerves. 1 – group 5x(5-HT+tet). 2 – group NaB+5tet+1x5-HT. 3– group TSA+5tet+1x5-HT. 4 – group NaB+5tet. 5 – group TSA+5tet. 6 – group 5tet. 7 – group 5tet+1x5-HT. For every neuron the responses at time point -40 min (left panel I), 60 minutes after the first tetanus (middle panel II), 120 min after the last tetanic stimulation (middle panel III), and 230 min after the last tetanic stimulation (right panel IV) are shown. Scale bars=5 mV, 500 ms