CHAPTER 4

POLY(ADP)-RIBOSE POLYMERASE ACTIVITY IS REQUIRED FOR HISTONE DEACETYLASE INHIBITOR-INDUCED ENHANCEMENT OF LONG-TERM MEMORY

INTRODUCTION

Histone Modifications

In the nucleus, DNA is packaged into chromatin. Nucleosome, the repeating unit of chromatin, consists of DNA wrapped around the histone octamer, which are made up of 2 copies of histones H2A, H2B, H3 and H4. Histone H1, the linker histone, associates with DNA between the two nucleosomes resulting in ‘solenoid’ helical fibers. The tails of histones, which protrude out of the nucleosomal assembly, serve as sites for multiple posttranslational modifications (Berger, 2007; Kouzarides, 2007). Distinct patterns of these modifications serve as histone 'language' or ‘code’ that regulates transcription.

Most of the histone modifications occur on lysine residues. The well characterized modifications include acetylation, methylation, ubiquitination, ADP-ribosylation and SUMOylation. Acetylation is associated with transcriptional activation. Methylation may be activating or inhibitory for transcription depending upon the specific residues that are modified (Strahl and Allis, 2000). Phosphorylation is another important histone modification, which typically occurs on serine, threonine or tyrosine residues. Phosphorylation of histone H3 at serine 10 is associated with the expression of immediate early genes like c-jun and c-fos. Histones also get methylated at arginine residues. Addition of more than one moiety on the same target residue may also occur. A single lysine residue could get mono-, di- or trimethylated (Strahl and Allis, 2000; Berger, 2007; Lee et al., 2010).

Histone Acetylation in Memory Formation

The importance of histone acetylation in synaptic plasticity and memory has been discussed earlier. However, since this Chapter of my thesis also involves histone acetylation, I will briefly discuss the role of this modification in memory. It is clear that in addition to the packaging function, histone modifications regulate transcription. Long-term memory (LTM) requires synthesis of RNA. Acetylation of histones neutralizes the positive charge of lysine and thus, decondenses the chromatin facilitating access to the transcriptional machinery. Considering that histone acetylation is facilitatory for transcription, it has been extensively studied with respect to memory formation.

Histone acetylation is regulated after memory training (e.g. Levenson et al., 2004). Several studies have used deacetylase inhibitors to increase acetylation level in the cells and study its effect on memory formation. Histone deacetylase (HDAC) inhibitors enhance memory formation in fear conditioning (Fischer et al., 2007; Lattal et al., 2007; Vecsey et al., 2007, Bredy and Barad, 2008), Morris water maze task (Fischer et al., 2007) and object recognition task (Stefanko et al., 2009). Wood and colleagues found that a training paradigm that normally does not induce LTM, was able to induce LTM in presence of HDAC inhibitor (Stefanko et al., 2009). Since inhibition of HDACs facilitates synaptic plasticity and memory, they behave as memory suppressor genes (Abel et al., 1998; Vecsey et al., 2007). Indeed, Guan and colleagues showed that HDAC deficiency enhances memory formation (Guan et al., 2009).

Poly(ADP)-Ribosylation of Histones

Many proteins including histones are post-translationally modified through addition of ADP-ribose moieties (Schreiber et al., 2006). This modification is carried out by poly(ADP)-ribose polymerase (PARP). These enzymes catalyze the transfer of ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to the lysine residues of target proteins, making them mono- or poly(ADP)-ribosylated. This modification also plays a pivotal role in chromatin remodeling and gene expression (Tulin and Spradling, 2003). PARP-1 is the most extensively studied member of the PARP family. Activation of PARP-1 was considered one of the earliest responses of cells in acute stress conditions, where it facilitates repair of damaged DNA strands. However, recent reports suggest that PARP-1 may have roles unrelated to DNA repair. For example, KCl-induced membrane depolarization and neurotrophins induce PARP-1 activity (Homburg et al., 2000; Visochek et al., 2005). PARP-1 modifies both linker histone H1 and core histones including H2B (Huletsky et al., 1989). Addition of the bulky ADP-ribose moieties, results in chromatin relaxation that facilitates recruitment of transcriptional machinery (D'Amours et al., 1999; Tulin and Spradling, 2003). PARP-1 may associate with enhancer and promoter regions also (D'Amours et al., 1999). Thus, PARP-1 may regulate transcription by promoting transcription factor binding and assembly of enhanceosome-like complexes (Kraus and Lis, 2003).

Poly(ADP)-Ribosylation in Memory Formation

Cohen-Armon and colleagues (Cohen-Armon et al., 2004) have studied the role of PARP-1 in synaptic plasticity and memory in *Aplysia*. They found that a pattern of serotonin application that induces long-term facilitation (LTF) of sensory-motor synapses, induces

poly(ADP)-ribosylation and thus, activation of PARP-1. Stimulus inducing short-term facilitation does not affect PARP activity. In addition, noxious stimuli that induce long-term sensitization cause poly(ADP)-ribosylation of PARP-1. In an operant-conditioning task, the authors showed that training in this task is associated with an increase in poly(ADP)-ribosylation of PARP-1. Moreover, they showed that inhibiting PARP-1 activity blocks LTM, but not short-term memory. Another study showed that PARP activity is required for LTF in *Aplysia* (Hernandez et al., 2009).

Studies in mammals also show that poly(ADP)-ribosylation is an important modification during LTM formation. Goldberg and colleagues showed that training in the object recognition task, increases poly(ADP)-ribosylation of PARP-1 and histone H1 (Goldberg et al., 2009). Inhibiting PARP activity blocks LTM formation in object recognition and fear conditioning task (Goldberg et al., 2009; Fontan-Lozano et al., 2010). Collectively, these studies suggested that poly(ADP)-ribosylation of proteins plays an important role in synaptic plasticity and memory formation in invertebrates as well as vertebrates.

Histone Acetylation and Poly(ADP)-Ribosylation

Previous studies have shown that PARP-1 promotes neurotrophin-induced acetylation of histone H4 (Cohen-Armon et al., 2004). Additionally, acetylated histones get preferentially poly(ADP)-ribosylated (Golderer and Grobner, 1991). These studies indicate a cross-talk between these two important modifications. As stated earlier, histone deacetylase inhibitors facilitate memory formation and, PARP-1 activity is required for memory formation. However, it is unclear whether poly(ADP)-ribosylation has a role in histone deacetylase inhibitor-induced facilitation of memory. In this Chapter, we show that sodium butyrate, a

histone deacetylase inhibitor, increases poly(ADP)-ribosylation of histone H1 in the hippocampus. In the presence of TiQ-A, a specific PARP inhibitor, sodium butyrate fails to increase poly(ADP)-ribosylation of histone H1. Finally, we show that PARP activity is required for beneficial effects of sodium butyrate on LTM formation.

MATERIALS AND METHODS

Animals

Two-to-three months old Sprague Dawley rats weighing 250-350 g were used for the experiments. Each rat was housed separately on 12 h/12 h light/dark cycle. The rats were given free access to rodent chow and water. All behavioral experiments were conducted between 6 p.m. and 10 p.m.

Object Recognition Task

The object recognition task was carried out as described in Chapter 1. Briefly, after habituation to the arena for 4 days, the animals were trained by allowing them to explore two copies of an object for 5 min or 10 min (depending upon the experiment as described below). The objects were kept on two corners of the arena. The memory for the object was tested 1 h and 24 h after training to test short-term memory (STM) and long-term memory (LTM), respectively. The same set of rats were used to test STM as well as LTM. During the memory test, one copy of the familiar object was replaced with a new object, and the rats were allowed to explore them for 5 min. The preferential exploration of the new object over the object used during training is an indication of memory formation for the object used during training. The activities of the animals were recorded using an overhead video camera. The time of interaction with the objects was analyzed manually. The time of interaction with the old object (T_{old}) and new object (T_{new}) were used to calculate the discrimination index, DI (Stefanko et al., 2009).

$$DI = \frac{(T_{new} - T_{old})}{(T_{new} + T_{old})} \times 100$$

To determine the effect of training on poly(ADP)-ribosylation of histone H1, rats were given object recognition training where they explored the two copies of an object for 10 min (Fontan-Lozano et al., 2010). One hour after training, rats were killed according to the procedure approved by Institutional Animal Ethics Committee, and their hippocampi were processed for analysis.

To determine the role of PARP-1 in LTM formation, rats received intraperitoneal injection of the PARP inhibitor, TiQ-A, 30 min before training. During training, the rats were exposed to two copies of an object for 10 min. One hour and 24 h after training, rats were tested for STM and LTM, respectively.

To study the effects of HDAC inhibition on memory, sodium butyrate was injected intraperitoneally 1 h before subjecting the rats to memory training. During training, the rats were exposed to the two copies of an object for 5 min. One hour and 24 h after training, rats were tested for STM and LTM, respectively.

To determine the interdependence of histone acetylation and poly(ADP)-ribosylation, the effect of PARP-1 inhibitor on sodium butyrate-induced facilitation of LTM was examined. For these experiments, rats received the PARP-1 inhibitor, TiQ-A, intraperitoneally. Thirty min after TiQ-A injection, they were given intra-peritoneal injection of histone deacetylase inhibitor, sodium butyrate. An hour after sodium butyrate injection, the rats were subjected

to memory training in which they were exposed to the two copies of an object for 5 min. One hour and 24 h after training, rats were tested for STM and LTM, respectively.

Drug Administration

Sodium butyrate is a widely used HDAC inhibitor that has been shown to enhance memory formation and rescue memory deficits. The rats were intraperitoneally injected with sodium butyrate (1.2 g/kg body weight; Stefanko et al., 2009). A stock solution of 120 mg/ml of sodium butyrate was prepared in normal saline. On an average, the rats received 2.5-3.5 ml of sodium butyrate solution. Control rats uniformly received 3 ml of normal saline.

The PARP-1 inhibitor, Thieno (2-3,c) isoquinolin-5-one (TiQ-A) was used to assess the role of poly(ADP)-ribosylation in LTM formation. The rats were intraperitoneally injected with TiQ-A (0.5 mg/Kg body weight; Fontan-Lozano et al., 2010). A stock solution of TiQ-A was prepared at 75 mM in DMSO. On an average, rats received 7-9 μl of this solution mixed with 493-491 μl of normal saline. Control rats uniformly received 8 μl of vehicle (DMSO) mixed with 492 μl of normal saline.

Nuclear Extract Preparation

The hippocampi of rats were harvested in Triton-X100 containing lysis buffer (Triton-LB: 50 mM Tris-Cl pH 7.5, 137.5 mM NaCl, 10% glycerol, 50 mM NaF, 1 mM Na_3VO_4, 1 mM sodium butyrate, 5 mM EDTA, protease inhibitor cocktail and 0.5% Triton-X100). The tissue was homogenized using a Dounce homogenizer (VWR) and kept on ice for 30 min with intermittent vortexing. The lysate was centrifuged at 100g for 5 min to remove the un-lysed cells. The supernatant was further centrifuged at 1500g for 15 min. The pellet was re-

suspended in Triton-LB and centrifuged again at 1500g for 15 min. The resulting pellet was collected as the nuclear fraction. This pellet was lysed in SDS-lysis buffer (SDS-LB: 50 mM Tris pH 7.5, 150 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4, 10 mM sodium butyrate, 1 mM EDTA, 2% sodium dodecyl sulfate (SDS) and protease inhibitor cocktail) and centrifuged at 14,000g for 10 min. The supernatant was collected as hippocampal nuclear lysate.

Isolation of Histone H1

The nuclei from the hippocampal tissue were isolated as described above. For isolation of histone H1, salt extraction procedure was used. The nuclear pellet was suspended in 0.5 M NaCl solution containing 0.2 mM EDTA. The suspension was incubated on ice for 30 min with intermittent vortexing, after which the suspension was centrifuged at 14,000g for 10 min to remove core histones and nuclear debris as pellet. The supernatant contained histone H1 protein.

Protein content in the nuclear and H1 extract was estimated using bicinchoninic acid (BCA, Pierce Biotechnology) with bovine serum albumin (BSA) as standard.

Western Blotting

Equal amount of protein from different samples was mixed with sample buffer (5X sample buffer: 250 mM Tris pH 6.8, 11.5% SDS, 40% glycerol, 25% β-mercaptoethanol, 0.5% bromophenol blue) and boiled for 5 min. The samples were resolved on polyacrylamide gel with 15% resolving and 4% stacking gels. Using wet transfer method, proteins were transferred to nitrocellulose membrane (MDI) at 200 mA for 90 min. After transfer, the membranes were washed with Tris-buffered saline containing Tween-20 (TBST: 25 mM

Tris-Cl pH 7.5, 137 mM NaCl, 2.7 mM KCl and 0.1% Tween-20). Blocking was done at room temperature for 1 h in 3% BSA in TBST. The membranes were then incubated with primary antibodies at 4^{o}C overnight. Before incubating the blots with secondary antibody, the membranes were washed with TBST. Horse radish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology) was diluted in blocking buffer and the incubation was carried out at room temperature for 1 h. After this incubation, the blots were washed again and the signals were detected using enhanced chemiluminescence reagent (Pierce). The blots were first probed with histone H1 antibody, which was stripped off before probing the blots with antibody against poly(ADP)-ribose. The antibody stripping was performed by incubating the blots in stripping buffer (62.5 mM Tris-Cl pH 6.8, 2% SDS, 100 mM β-mercaptoethanol) at 65^{o}C for 2 h with a change of buffer after 1 h of incubation. Stripping of antibody was confirmed by probing the blot with secondary antibody alone. The signals were captured on Hyperfilm (Amersham). Band intensities were quantified using NIH Image (NIH, Bethesda).

Antibodies

Antibodies that were used for the study are: anti-histone H1 (Active Motif), and anti-poly(ADP)-ribose (Calbiochem) and HRP-conjugated secondary antibody (Cell Signaling Technology).

Data Analysis

For the analysis of histone H1 poly(ADP)-ribosylation, the poly(ADP)-ribose signal of histone H1 was divided by total histone H1 signal in each lane and then normalized with the control sample. Data were analyzed using unpaired student's t-test on ratios of poly(ADP)-

ribose signal to total histone H1 signal. The effect of TiQ-A on sodium butyrate-induced poly(ADP)-ribosylation of histone H1 was analyzed using analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hoc analysis.

The effect of sodium butyrate or TiQ-A on memory formation was analyzed using student's t-test. Within group analysis was done using one sample t-test on discrimination index. A paired t-test on the time spent exploring the old and new objects gave similar results. Analysis between groups was done using unpaired t-test on the discrimination index. The effect of TiQ-A on sodium butyrate-induced LTM was analyzed using ANOVA on the discrimination index, followed by Student-Newman-Keuls post-hoc analysis. Results were considered significant when p value was < 0.05. All data are expressed as mean $\pm$ SEM.

RESULTS

Our main aim in this study was to examine interaction between poly(ADP)-ribosylation and acetylation in memory formation. Before conducting these experiments, we tried to reproduce some of the previous findings on the role of poly(ADP)-ribosylation and acetylation in memory formation.

Memory Training Increases Poly(ADP)-Ribosylation of Histone H1

Object recognition training increases poly(ADP)-ribosylation of histone H1 in the hippocampus (Fontan-Lozano et al., 2010). We trained the animals in this task and examined histone H1 poly(ADP)-ribosylation. A schematic depiction of the experiment is shown in Figure 1. Rats were divided into two groups – control and trained. Both the groups were habituated to the arena. Rats in the trained group were exposed to two copies of an object for 10 min. Rats in the control group were exposed to the arena without any object, for equal duration. An hour after training, the rats were killed and their hippocampi were isolated and examined for changes in poly(ADP)-ribosylation of histone H1. Result showed that poly(ADP)-ribosylation of histone H1 was significantly increased in the hippocampi of the trained rats compared to the habituated controls (Fig. 2). In addition to being consistent with the previous finding (Fontan-Lozano et al., 2010), the result shows that histone H1 poly(ADP)-ribosylation lasts for at least 1 h after memory training

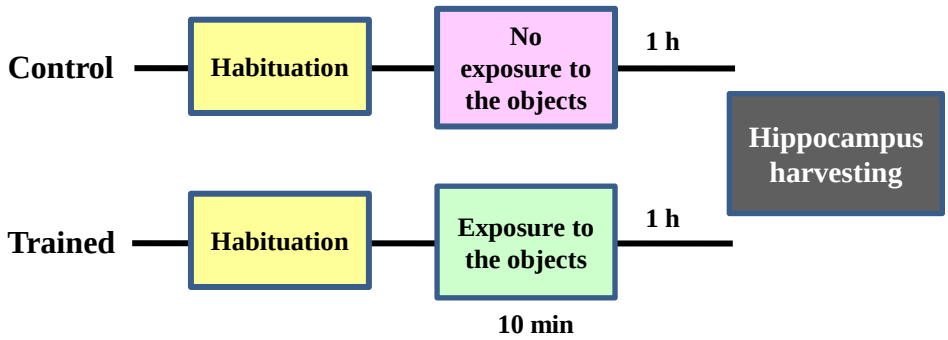

Figure 1. Schematic depiction of the experiment designed to study the effect of training on poly(ADP)-ribosylation of histone H1. Rats in the control and trained groups were habituated to the arena. During training, the animals were exposed to two copies of an object for 10 min. This training paradigm forms long-term memory that persists for at least 24 h. The control group was exposed to the arena for 10 min without any object. One hour after training, rats in both the groups were killed and their hippocampi were harvested for histone H1 analysis.

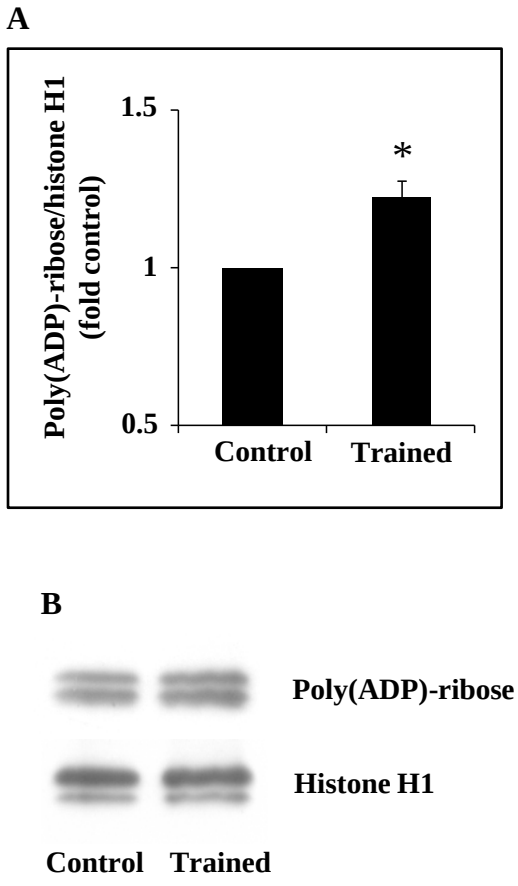

Figure 2. Memory training increases poly(ADP)-ribosylation of histone H1. Nuclear extracts prepared from the hippocampi of control and trained rats were probed with poly(ADP)-ribose and histone H1 antibodies. The quantified summary of data (A, n = 9) and the representative Western blots (B) show that memory training increased poly(ADP)-ribosylation of histone H1 in the hippocampus. Asterisk denotes significant difference ($p < 0.05$).

Histone Deacetylase Inhibitor Increases Poly(ADP)-Ribosylation of Histone H1 in the Hippocampus

Earlier studies have shown that poly(ADP)-ribosylation occurs preferentially on acetylated histone H4 than non-acetylated histone H4. Importantly, histone deacetylase inhibitor, sodium butyrate, increases mono(ADP)-ribosylation of histone H4 (Boulikas, 1990; Golderer and Grobner, 1991). We examined whether enhancing histone acetylation through HDAC inhibition increases poly(ADP)-ribosylation of histone H1. Intraperitoneal injection of HDAC inhibitor, sodium butyrate, increased poly(ADP)-ribosylation of histone H1 in the hippocampus (Fig. 3A and B). Although sodium butyrate increased poly(ADP)-ribosylation of proteins in addition to histone H1, we focused our study on histone H1 because a role of histone H1 poly(ADP)-ribosylation has been proposed in LTM formation (Fontan-Lozano et al., 2010).

The above experiments were conducted on hippocampal nuclear extracts. In another set of experiment, we isolated histone H1 and examined its poly(ADP)-ribosylation. Again, the result showed that sodium butyrate increased poly(ADP)-ribosylation of histone H1 (Fig. 3C).

PARP-1 Activity is Required for HDAC Inhibitor-Induced Poly(ADP)-Ribosylation of Histone H1

To examine the role of PARP-1 activity in HDAC inhibitor-induced increase in poly(ADP)-ribosylation of histone H1, we used a specific PARP-1 inhibitor, TiQ-A. Although the rats injected with sodium butyrate showed increased poly(ADP)-ribosylation of histone H1, in the presence of TiQ-A, sodium butyrate failed to increase histone H1 poly(ADP)-

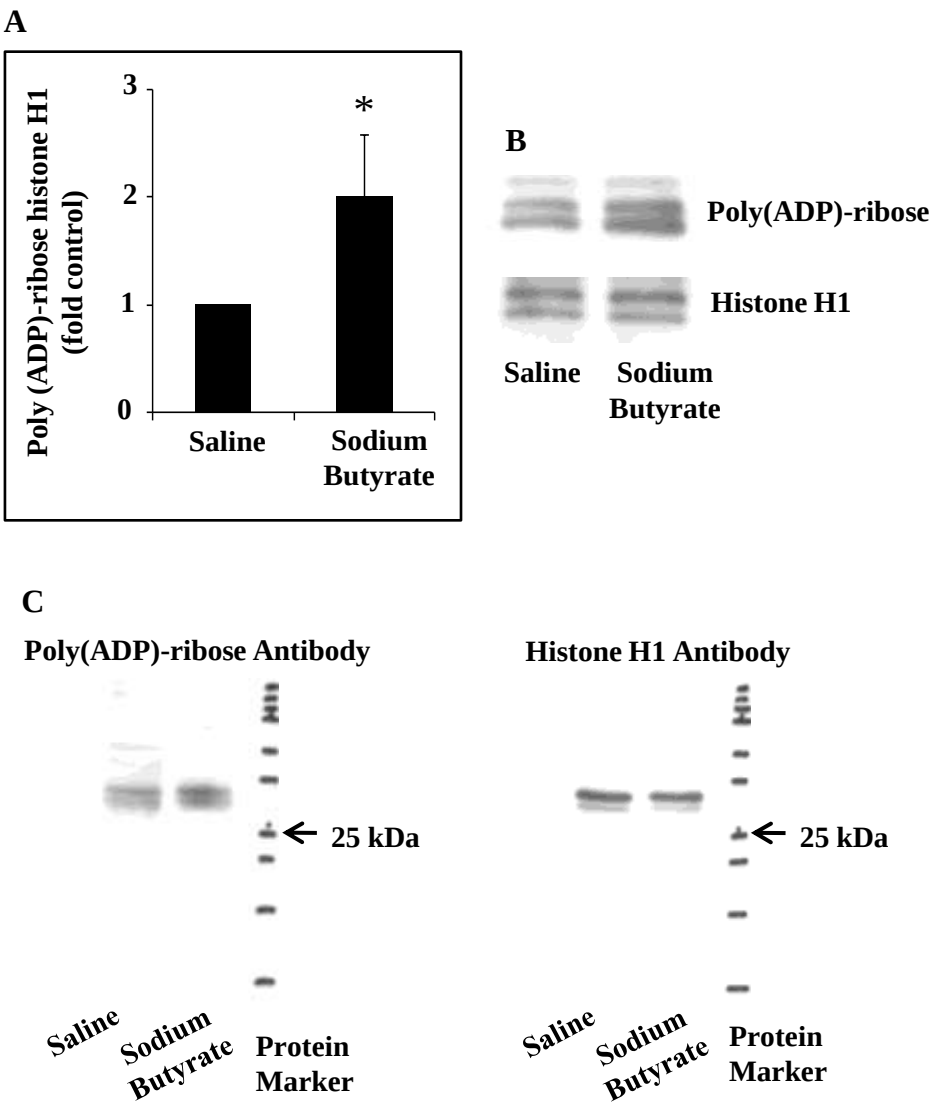

Figure 3. Histone deacetylase inhibition increases poly(ADP)-ribosylation of histone H1 in the hippocampus. Nuclear extracts prepared from the hippocampi of saline- and sodium butyrate-injected rats were probed with poly(ADP)-ribose and histone H1 antibodies. The quantified summary of data (A, n = 5) and the representative blots (B) show that sodium butyrate increased poly(ADP)-ribosylation of histone H1 in the hippocampus. Asterisk denotes significant difference ($p < 0.05$). Histone H1 poly(ADP)-ribosylation was examined using isolated histone H1 from hippocampi of saline and sodium butyrate-injected animals (C). The Western blots show that sodium butyrate increased poly(ADP)-ribosylation of histone H1.

ribosylation (Fig. 4). The result suggests that in addition to inhibiting histone deacetylases, sodium butyrate also affect PARP activity.

Blocking PARP Activity Inhibits LTM Formation

The molecular analyses described in the previous sections show that there exists a cross-talk between acetylation and poly(ADP)-ribosylation. We next focused on interaction between these two modifications in memory formation after replicating previous relevant results on the role of PARP and acetylation on memory formation.

As shown in previous studies (Stefanko et al., 2009) and Chapter 1 (Fig. 17A-C), training in object recognition task leads to memory of the object that persists for at least 24 h. We examined the requirement of poly(ADP)-ribosylation in memory formation. The rats were injected with the PARP-1 inhibitor, TiQ-A, and then trained in the object recognition task where they were exposed to two copies of an object for 10 min. We found that during test for STM, saline and TiQ-A injected rats explored new object more than the old object (Fig. 5A) indicating memory for the old object. Although during the STM test, TiQ-A-injected rats showed slightly larger discrimination index than saline-injected rats (47.59 versus 32.65), the difference was not statistically significant.

During the LTM test, saline-injected rats explored the new object more than the familiar object indicating that the animals developed LTM for the objects presented during training. On the contrary, TiQ-A-injected rats did not show any preference for the new object (Fig. 5B). Thus, consistent with earlier findings (Goldberg et al., 2009; Fontan-Lozano et al., 2010), our results show that PARP activity is required for LTM formation.

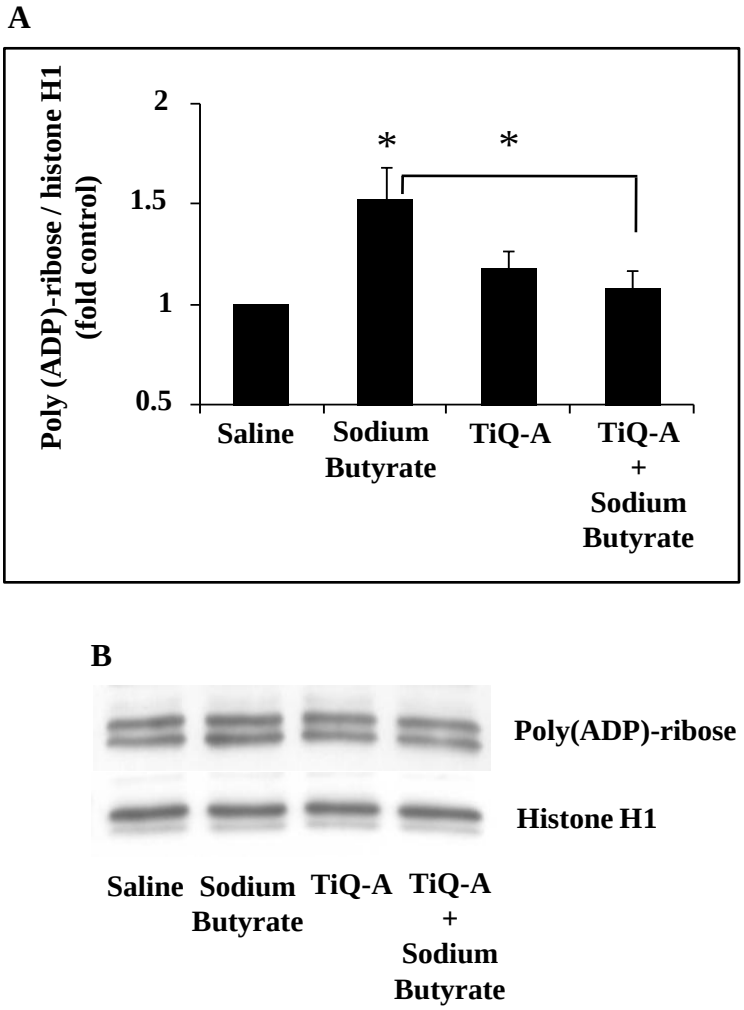

Figure 4. Sodium butyrate-induced poly(ADP)-ribosylation of histone H1 requires PARP activity. Nuclear extracts prepared from the hippocampi of saline-, sodium butyrate-, TiQ-A- or TiQ-A + sodium butyrate-injected rats were probed with poly(ADP)-ribose and histone H1 antibodies. The quantified summary of data (A, n = 6) and the representative blots (B) show that TiQ-A blocked sodium butyrate-induced increase in poly(ADP)-ribosylation of histone H1 ($F_{3, 23}$ = 3.45, p = 0.036; saline versus sodium butyrate, p = 0.04 and sodium butyrate versus TiQ-A + sodium butyrate, p = 0.04). TiQ-A alone did not show any effect on histone H1 poly(ADP)-ribosylation. Asterisks denote significant difference.

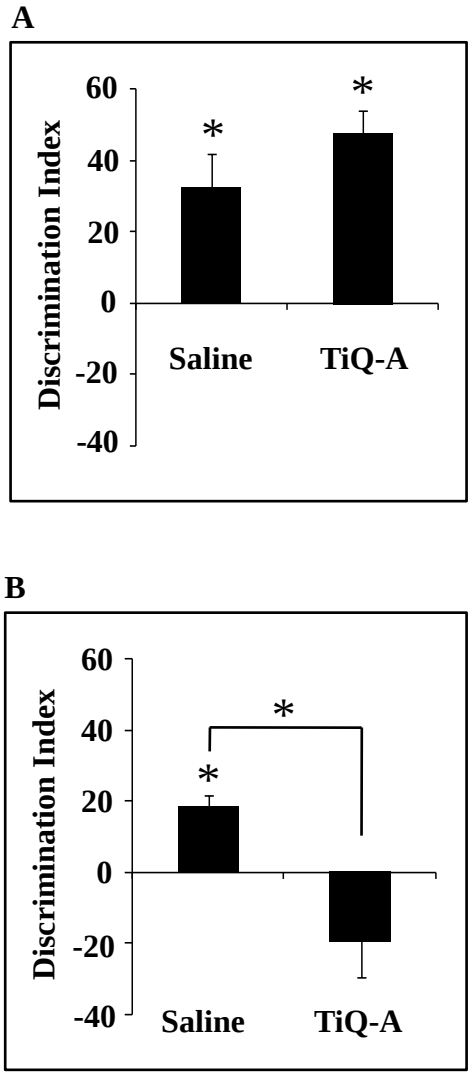

Figure 5. PARP inhibitor blocks LTM formation. Rats injected with saline or the PARP inhibitor, TiQ-A, were subjected to object recognition training. (A) During test for STM, saline- or TiQ-A-injected rats preferentially interacted with the new object than the old object and showed comparable discrimination indices. (B) During test for LTM, saline-injected rats preferred interacting with the new object showing LTM for the old object. However, the TiQ-A-injected rats did not show any preference for the new object indicating lack of LTM. Same set of animals (n = 5) were used for STM and LTM. Asterisks denote significant difference ($p < 0.05$).

Histone Deacetylase Inhibition Causes a Weak Training to Form LTM

Stefanko and colleagues have previously shown that in object recognition task, HDAC inhibition can lead to LTM formation with a sub-threshold training (Stefanko et al., 2009). We reproduced these findings under our conditions. As depicted in Figure 6A, sodium butyrate- or normal saline-injected rats were given a 5-min exposure to two copies of an object during training and subsequently tested for LTM. During LTM test, the saline-injected rats equally explored both new and old objects whereas the sodium butyrate injected rats explored the new object more than the old object. Sodium butyrate-injected rats presented significantly larger discrimination index than saline-injected animals (Fig. 6B). These results are consistent with the earlier findings where sodium butyrate has been shown to form LTM after a weak training (Fontan-Lozano et al., 2008; Stefanko et al., 2009).

PARP Activity is Required for Histone Deacetylase Inhibitor-Induced Facilitation of LTM Formation

Having replicated the earlier findings that PARP activity is required for LTM formation and HDAC inhibition enhances LTM, we next asked whether blocking PARP activity affects HDAC inhibitor-induced LTM formation.

To investigate the role of PARP-1 activity in deacetylase inhibitor-induced LTM, rats injected with sodium butyrate, in presence or absence of TiQ-A, were subjected to a 5-min (weak) memory training. The animals were tested for STM (1 h after training) and LTM (24 h after training). A schematic depiction of the experiment is shown in Figure 7. We found that during test for STM, rats in all the groups presented similar discrimination indices (Fig. 8A). During LTM test, sodium butyrate-injected rats explored the new object more than the

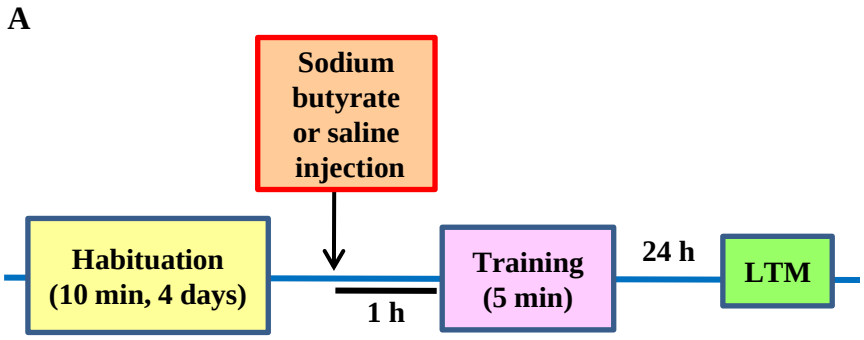

Figure 6A. Schematic depiction of the experiment designed to study the effect of sodium butyrate on weak training. Rats received intraperitoneal injection of sodium butyrate or saline 1 h before a 5-min exposure to two copies of an object (weak training). The rats were subjected to test for STM 1 h after training. LTM test was conducted 24 h after training.

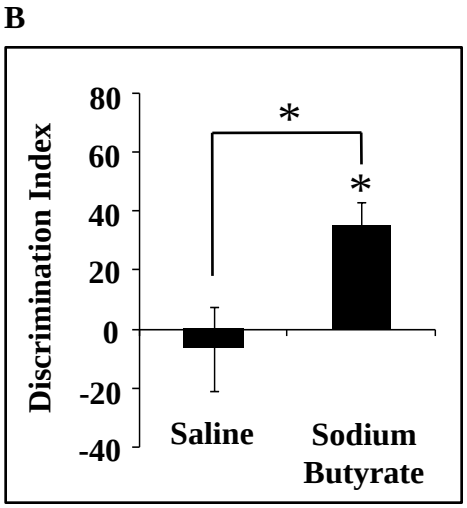

Figure 6B. Sodium butyrate causes weak training to form LTM. Rats injected with saline or sodium butyrate were given weak memory training. During test for LTM, the sodium butyrate-injected rats preferred interacting with the new object whereas the saline-injected rats did not show preference for the new object. The sodium butyrate-injected rats showed significantly higher discrimination index compared to the saline-injected controls (n = 5, both groups). Asterisks denote significant difference ($p < 0.05$).

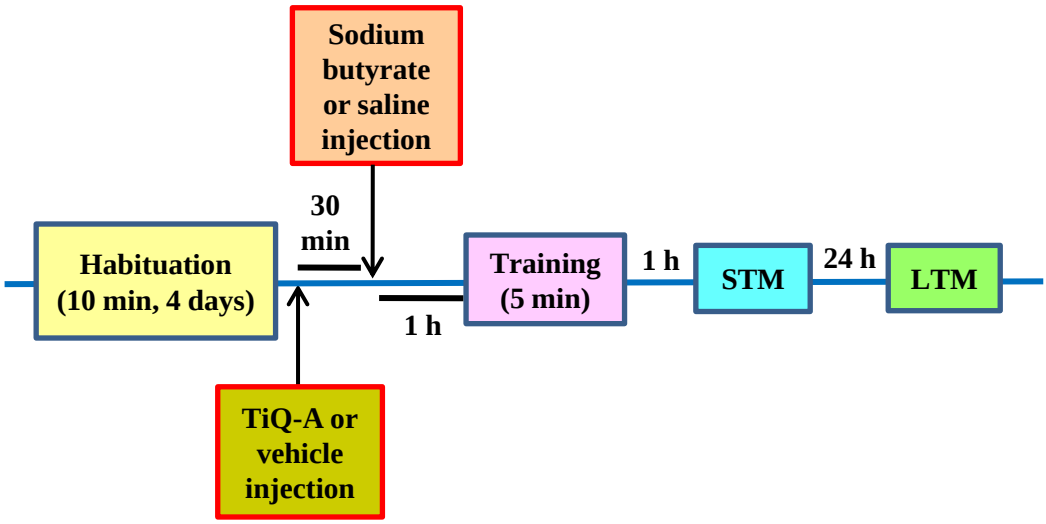

Figure 7. Schematic depiction of the experiment designed to study the effect of PARP inhibitor on deacetylase inhibitor-induced facilitation of long-term memory. The animals were habituated to the arena. On the day of training, rats received TiQ-A or vehicle injection. Thirty min later, they were injected with sodium butyrate or saline. An hour later, rats were trained by exposing them for 5 min (weak training) to two copies of an object. STM and LTM tests were performed 1 h and 24 h after training, respectively.

old object, showing larger discrimination index. But in the presence of TiQ-A, sodium butyrate-injected rats (TiQ-A + sodium butyrate) showed similar exploration of new and old objects (Fig. 8B). PARP inhibition alone had no effect on LTM formation. These results show that HDAC inhibitors recruit PARP activity to bring about facilitatory effects on memory formation.

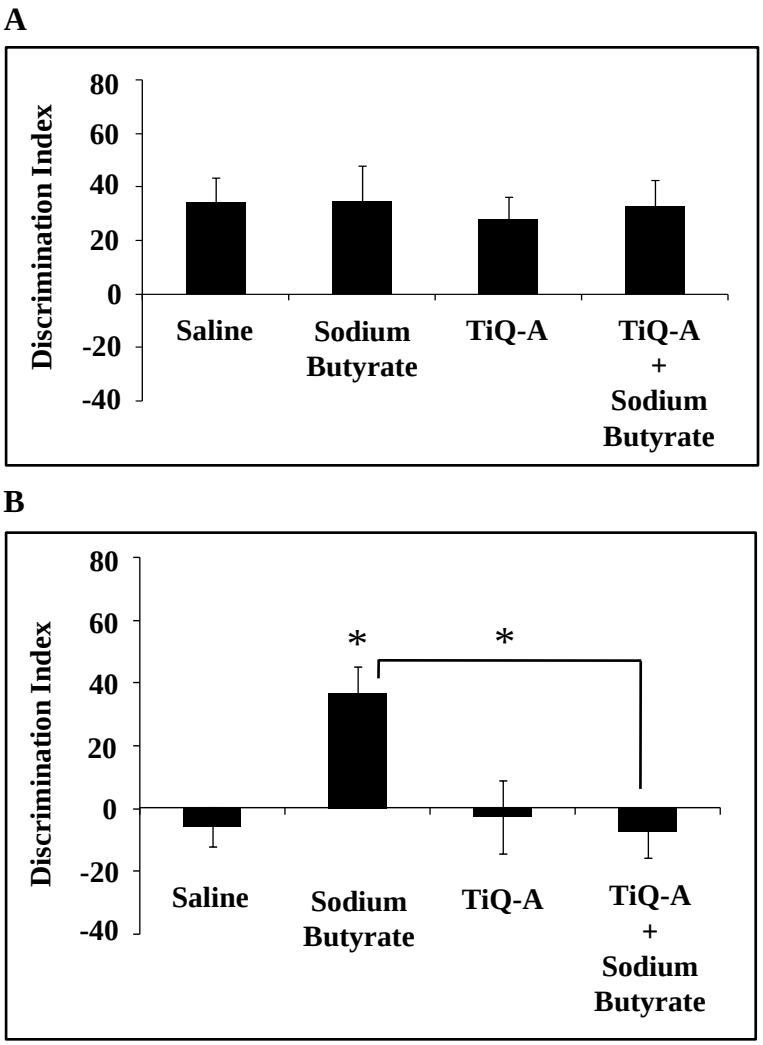

Figure 8. PARP activity is required for sodium butyrate-induced LTM formation after weak training. Saline, sodium butyrate, TiQ-A or TiQ-A + sodium butyrate-injected rats were subjected to a weak object recognition training (Fig. 8) that does not form LTM. (A) During test for STM, all the groups preferentially interacted with the new object than the old object resulting in similar discrimination indices. There was insignificant difference between groups ($F_{3, 31} = 0.08$, $p = 0.97$). (B) During test for LTM, sodium butyrate-injected rats explored the new object more than the old object (higher discrimination index) showing memory for the object used during training. However, TiQ-A inhibited the facilitatory effect of sodium butyrate on LTM formation ($F_{3, 31} = 5.8$, $p = 0.003$; saline versus sodium butyrate, $p = 0.005$, sodium butyrate versus TiQ-A + sodium butyrate, $p = 0.007$). TiQ-A alone did not show any effect on LTM. Same set of animals (n = 8) were used for STM and LTM. Asterisks denote significant difference ($p < 0.05$).

DISCUSSION

A wealth of literature has demonstrated that histone acetylation is an important event in synaptic plasticity and memory formation. Studies have also shown that poly(ADP)-ribosylation of proteins is a critical event in these processes. However, it is unclear whether there exists any cross-talk between these two modifications in synaptic plasticity and memory. Chapter 4 of my thesis has examined this important aspect. We first established the previous findings that were critical to examine interaction between the two modifications.

A previous study has shown that training in object recognition task enhances poly(ADP)-riboslyation of histone H1 (Fontan-Lozano et al., 2010) immediately after training. We examined histone H1 poly(ADP)-ribosylation 1 h after training and found that it was enhanced. Collectively, these results show that histone H1 poly(ADP)-ribosylation induced by memory training lasts for at least 1 h. The longer duration of this modification may regulate gene transcription for a longer time and contribute to synaptic plasticity and memory.

During the study on effects of PARP inhibitor on memory formation, we found that although the PARP inhibitor-injected animals presented higher discrimination index compared to saline controls during the STM test, the difference was not statistically significant suggesting that PARP activity is not required for STM formation. Fontan-Lozano and colleagues have shown that PARP-1 inhibitor, TiQ-A, does not affect STM, tested 1 h after training (Fontan-Lozano et al., 2010). However, another study found that PARP-1 inhibitor, PJ-34, improves STM, tested 30 min after training (Goldberg et al., 2009). Our results are similar to the results of Fontan-Lozano and colleagues. With regards to LTM formation, however, our

studies and the studies referred above show that inhibition of PARP-1 blocks LTM, tested 24 h after training. Thus, poly(ADP)-ribosylation is an important event in long-term memory formation.

Earlier studies have shown that HDAC inhibition transforms a transcription-independent form of memory to a transcription-dependent form of memory (Stefanko et al., 2009). The authors found that exposing mice to objects for 3 min does not form LTM. However, with inhibition of deacetylases, the animals form long-lasting memory. Similar results were obtained in a study by Fontan-Lozano et al. (2008) using a 5-min exposure to the objects during training. Here, we found that a single 5 min exposure to the objects is not sufficient to form LTM. However, when the rat received 5 min exposure, after an injection of sodium butyrate, they formed LTM for the object. Thus, the results are consistent with earlier findings that HDAC inhibition lowers the threshold of consolidation processes required for LTM formation (Fontan-Lozano et al., 2008).

With regards to ribosylation and acetylation cross-talk, *in vitro* studies have shown that acetylated histone is a preferred substrate for poly(ADP)-ribosylation (Golderer and Grobner, 1991). We found that HDAC inhibition increases level of poly(ADP)-ribosylated histone H1 in the intact animal. Importantly, the deacetylase inhibitor-induced poly(ADP)-ribosylation of histone H1 requires PARP activity. These results raised the possibility that sodium butyrate-induced poly(ADP)-ribosylation may be important for memory formation. In a weak object recognition training, we found that as expected, the sodium butyrate injected group formed LTM of the familiar object. However, the LTM formed with HDAC inhibition was blocked by PARP inhibitor. Collectively, these results suggest an interaction between two histone modifications, acetylation and poly(ADP)-ribosylation, in regulating long-term

memory formation. It would be interesting to explore how cross-talk between these histone modifications affects the expression of genes important for long-term memory.

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PUBLICATION

Pandey K., Sharma S.K. (2011) Activity-dependent acetylation of alpha tubulin in the hippocampus. **Journal of Molecular Neuroscience** 45:1-4