



The histone H3 lysine 9 methyltransferase G9a/GLP complex activity is required for long-term consolidation of spatial memory in mice

Kyrian Nicolay-Kritter, Jordan Lassalle, Jean-Louis Guillou, N Mons

► To cite this version:

Kyrian Nicolay-Kritter, Jordan Lassalle, Jean-Louis Guillou, N Mons. The histone H3 lysine 9 methyltransferase G9a/GLP complex activity is required for long-term consolidation of spatial memory in mice. *Neurobiology of Learning and Memory*, 2021, 179, pp.107406. 10.1016/j.nlm.2021.107406 . hal-03422574

HAL Id: hal-03422574

<https://cnrs.hal.science/hal-03422574>

Submitted on 22 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**The Histone H3 Lysine 9 Methyltransferase G9a/GLP complex activity
is required for long-term consolidation of spatial memory in mice.**

Running title: Histone methylation and memory

Kyrian Nicolay-Kritter^{1,2}, Jordan Lassalle^{1,2}, Jean-Louis Guillou^{1,2} and Nicole Mons^{1,2}

¹ Université de Bordeaux, France. ² Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5287, 33615 Pessac, France.

Correspondence should be addressed to N. Mons. Université de Bordeaux, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5287, 33615 Pessac, France.

Email: nicole.mons@u-bordeaux.fr

Acknowledgements: The present study was supported by the CNRS, Université de Bordeaux and Observatoire B2V des memoires and PREVAAL Finance to K. Nicolay-Kritter. We would like to thank J. Lassalle for his valuable contributions to behavioural experiments, G. Courtand for guidance with the image analysis, T Lafon for technical assistance and E. Poinama for animals care.

Declaration of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the manuscript.

CRediT Authorship contribution statement

Kyrian Nicolay-Kritter: Investigation, Formal analysis, Methodology, Writing - original draft, Visualization. **Jordan Lassalle:** Investigation, Methodology, Formal analysis. **Jean-Louis Guillou:** Conceptualization, Formal analysis, Supervision, Writing - review & editing. **Nicole Mons:** Conceptualization, Funding acquisition, Supervision, Methodology, Writing - re-view&editing.

Abstract

The G9a/G9a-like protein (GLP) histone lysine dimethyltransferase complex and downstream histone H3 lysine 9 dimethylation (H3K9me2) repressive mark have recently emerged as key transcriptional regulators of gene expression programs necessary for long-term memory (LTM) formation in the dorsal hippocampus. However, the role for hippocampal G9a/GLP complex in mediating the consolidation of spatial LTM remains largely unknown. Using a water maze competition task in which both dorsal hippocampus-dependent spatial and striatum-dependent cue navigation strategies are effective to solve the maze, we found that pharmacological inhibition of G9a/GLP activity immediately after learning disrupts long-term consolidation of previously learned spatial information in male mice, hence producing cue bias on the competition test performed 24h later. Importantly, the inhibition of hippocampal G9a/GLP did not disrupt short-term memory retention. Immunohistochemical analyses revealed increases in global levels of permissive histone H3K9 acetylation in the dorsal hippocampus and dorsal striatum at 1h post-training, which persisted up to 24h in the hippocampus. Conversely, H3K9me2 levels were either unchanged in the dorsal hippocampus or transiently decreased at 15min post-training in the dorsal striatum. Finally, the inhibition of G9a/GLP activity further increased global levels of H3K9 acetylation while decreasing H3K9me2 in the hippocampus at 1h post-training. However, both marks returned to vehicle control levels at 24h. Together, these findings support the possibility that G9a/GLP in the dorsal hippocampus is required for the transcriptional switch from short-term to long-term spatial memory formation.

Keywords: Hippocampus; Striatum; Histone methylation; Learning; Memory

1. Introduction

In the last decade, emerging evidence emphasizes dynamic chromatin remodeling through post-translational modifications (PTM) of histone proteins as a key player of gene expression patterns underpinning the formation of long-term memories (LTM) (Peixoto & Abel, 2012; Stilling & Fischer, 2011; Sweatt, 2009). Most of the studies on the dynamic changes in histones PTM during learning and memory processes have focused on acetylation and methylation at multiple lysine residues on the N-terminal tail of specific histones. Studies in rodents have emphasized an essential role of histone acetylation in the dorsal hippocampus (dHPC) in activating the gene expression program necessary for LTM consolidation in several HPC-dependent paradigms, including contextual fear conditioning and spatial learning (Bousiges et al., 2010; Castellano et al., 2012; Castellano et al., 2014; Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007; Levenson et al., 2004; Peleg et al., 2010; Sweatt, 2009; Vecsey et al., 2007). We previously reported that local infusion of histone deacetylase inhibitor (HDACi) into the dHPC immediately after learning selectively enhances LTM consolidation of spatial water maze training by influencing the strength of functional connectivity between the hippocampus and interconnected structures (Dagnas, Guillou, Prevot, & Mons, 2013). While histone acetylation is a permissive transcriptional mark, changes in lysine methylation of histones are associated with either activation or repression of gene expression, depending on the location of methylated residue and, for a single lysine residue, the degree of methylation status. In recent years, dimethylation of histone H3 lysine 9 (H3K9me2), a repressive mark catalyzed by histone methyltransferases (HMT) G9a (also known as Ehmt2) and G9a-like protein (GLP, also known as Ehmt1), has emerged as a critical epigenetic regulator of HPC-dependent LTM formation and long-lasting forms of synaptic plasticity (for review, see (Benevento, van de Molengraft, van Westen, van Bokhoven, & Kasri, 2015; Pang, Sharma, & Sajikumar, 2019). Several studies have reported, for example, a pivotal role for

G9a/GLP-mediated H3K9me2 in mediating transcriptional homeostasis between gene activation and silencing in area CA1 of the dHPC (dCA1) and the entorhinal cortex during contextual fear memory consolidation (Gupta-Agarwal et al., 2012; Gupta et al., 2010; Mahan et al., 2012). Pharmacological inhibition or genetic silencing of G9a/GLP activity in the dCA1 impairs contextual fear LTM and disrupts hippocampal long-term potentiation (LTP) (Gupta-Agarwal et al., 2012; Gupta et al., 2010; Schaefer et al., 2009). In contrast, the same G9a/GLP blockade in the entorhinal cortex had the opposite effect on fear LTM while increasing both repressive H3K9me2 marks at promoter regions of non-memory permissive genes (e.g. *COMT*) and permissive H3K4me3 and H3K9ac marks at memory-related gene promoters (e.g. *Zif268*; *BDNF exon IV*; *DNMT3*) in the dHPC. Electrophysiology studies in rats also indicated that G9a/GLP complex activity exerts an epigenetic control over BDNF-mediated long-term plasticity and associativity in hippocampal CA1 neurons (Sharma, Dierkes, & Sajikumar, 2017). Collectively, these findings suggest that an accurate regulation of G9a/GLP-mediated H3K9me2 may play a pivotal role in maintaining a fine balance between gene activation and gene silencing during the process of LTM consolidation.

Dual solution tasks are used to study how multiple memory systems interact with one another over time and/or training regimen (Gasser, Pereira de Vasconcelos, Cosquer, Boutillier, & Cassel, 2020; Martel et al., 2007; Rice, Wallace, & Hamilton, 2015). Using a water maze competition task, in which both HPC-based spatial/place and striatum-based cue/response strategies are effective solutions to solve the task, we previously demonstrated that inducing histone hyperacetylation in the dorsal HPC immediately after learning operates as a molecular switch between the HPC- and dorsal striatum-dependent memory systems, biasing mice towards predominant use of a spatial navigational strategy under training conditions that normally results in the use of the cued strategy (Dagnas et al., 2013). The present study used the same water maze task to examine whether immediate post-training infusion of the

pharmacological G9a/GLP inhibitor, BIX-01294, administrated into the dHPC of male mice would also influence the consolidation of one particular memory strategies (i.e. spatial *versus* cue-guided) assessed 24h later in competition probe tests. Since a proper balance between transcriptionally active H3K9ac and repressive H3K9me2 marks *via* accurate regulation of the HMT G9a/GLP activity is important for coordinating patterns of gene expression during the process of memory consolidation, we also explored whether differences in performance in the BIX-infused mice would be accompanied by altered patterns of changes of the two histone marks in the dCA1 and the dorsal striatum. Taken together, our results suggest that G9a/GLP complex activity in the dHPC is essential for ensuring the coordinated expression of gene networks that are required for consolidation of long-term spatial memory.

2. Materials and Methods

2.1 Animals. Male C57BL/6 mice from Charles River Laboratories (L'Arbresle, France) at 4 months old at the beginning of the experiments were used. They were individually housed in a temperature-controlled colony room with a 12:12 hour light-dark cycle (lights on at 7:00 am) and were provided with food and water *ad libitum*. Behavioral tests were performed during the light cycle between 08:00 and 13:00 hour. In addition, all mice were handled for 5 days (5 min each time) before any experimental manipulation. All procedures were approved by the local Ethics Committee for Animal Experiments (N° 501-20-89) in compliance with the European Communities Council Directive of 1st February 2013 (2010/63/UE).

2.2 Surgery. Mice were anesthetized with a mixture of ketamine (100 mg / kg; body weight, i.p.) and xylazine (6 mg / kg; body weight, i.p.) (Kopf Instruments, Wuppertal, Germany) and placed in a stereotaxic apparatus (Kopf instruments). Stainless-steel guide cannulae (26 gauge, 8 mm length) were implanted bilaterally 1 mm above the dCA1 (AP -2; ML \pm 1.4; DV 0.9; mm from Bregma, (Franklin & Paxinos, 1997). Guide cannulae were fixed to the skull with dental cement and three jewel screws. Mice were given 10 days of recovery before water maze training was conducted.

2.3 Local drug infusion. The G9a/GLP HMT inhibitor BIX 01294 (BIX; Tocris Bioscience, Cat#3364) was stored at 10 mM stock in DMSO at -20°C. Bilateral injections of 0.5 μ L of BIX (45 μ mol; (Gupta-Agarwal et al., 2012) or its vehicle (aCSF) were infused into the dorsal HPC immediately or 1h after last training trial on Day 1. Bilateral infusion took ~ 5 min and cannulae were left in place for an additional 1 min before removal to allow diffusion of the drug away from the cannulae tips. Placement implantations were controlled using thionine blue coloration on 60 μ m coronal brain sections cut with a freezing microtome (Leica SM2400) and visualized under optical microscope (Fig. 1A).

2.4. Behavioral task.

2.4.1 Apparatus. An elevated circular pool (150 cm diameter x 55 cm height) was filled with water maintained ($21 \pm 1^\circ\text{C}$) that was rendered opaque with non-toxic white paint. Two Plexiglas platforms (PF, 13 cm diameter) were submerged 1 cm below the water surface. Several distal visual cues located on the walls of the experimental room served as oriental landmarks.

2.4.2 Procedure. Mice underwent a training session of 8 or 12 trials followed 1h or 24h later by a competition phase consisting of a series of three probe trials (Fig. 1A, (Martel et al., 2007)).

During the training day, the PF remained in a fixed position (northwest quadrant) and was marked with a 10 cm height striped cylindrical intra-maze cue allowing a balanced “spatial” and “non-spatial” version of the reference memory task. In each trial, mice were placed, facing the wall, at a constant start position (middle of the southeast quadrant). The trial was completed when the mouse found and climbed onto the cued PF or 90 s had elapsed. If a mouse failed to find the PF within 90 s, it was gently guided onto the PF by hand where it was left for 20 s. After each trial, mice were dried and returned to their home cage placed in a warm box equipped with dark lamps. Mice were trained in squads of 5 (10-12 min inter-trial interval) and fully counterbalanced with respect to groups.

The competitive test was conducted 1h or 24h after completion of the training phase and consisted of three test trials (5min inter-trial interval). During each trial, two PF were submerged to assess the search strategy used. A non-visible PF was placed in the original training location (northwest quadrant) whereas the cued-PF was moved to the opposite quadrant (southeast). The start position was equidistant to old and new quadrants location of the PF (southwest). If the animals swam to old quadrant (hidden) PF location, a place

response was noted; if the animal swam to the new quadrant (cued) PF location, a cue-guided response was noted.

2.4.3. Quantification and analysis of behavioral data. A video camera mounted above the pool was used to record swim trials. The data were recorded and analysed using an automated tracking system (Videotrack, Champagne au Mont d'Or, France). The acquisition performance was analyzed for each trial, defined as the mean latency (time (sec) to escape from the releasing point to the submerged platform). During the competitive test, performance was calculated as mean percentage of a particular (spatial *versus* cue) response or mean percentage of time spent in each half of the pool over the three test trials. In addition, an individual profile was categorized as place or cue-guided strategy according to the majority choice made by the animal over the three probe trials when responding to the old or new PF location, respectively.

2.5. Immunohistochemistry and quantification. Animals were deeply anesthetized with ketamine/xylazine (100mg/20mg/Kg, ip) and were transcardially perfused with ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed and post-fixed overnight, then sectioned (40 μ m) on a vibratome (Leica). Serial coronal sections were cryoprotected in 30% ethylene glycol and 30% glycerol in PB and stored at -20°C until processed for immunohistochemistry as previously described in detail (Dagnas et al., 2013; Dagnas, Micheau, Decorte, Beracochea, & Mons, 2015; Dagnas & Mons, 2013). Briefly, free-floating sections were incubated with rabbit polyclonal antibodies for H3K9me2 (1:5000, Millipore) or H3K9Ac (1:3000, Millipore). Then, sections were incubated with a biotinylated goat anti-rabbit IgG secondary antibody (1:2000; Jackson immunoresearch). This was followed by incubation with an avidin-biotinylated horseradish peroxidase complex (Vectastain Elite kit, Vector Laboratories, Burlingame, CA). The peroxidase reaction was visualized in a Tris

solution containing diaminobenzidine tetrahydrochloride and hydrogen peroxide. Sections were mounted on gelatine-coated slides, dehydrated and coverslipped. All images were acquired using an imaging analysis system (Biocom Visiolab 2000, V4.50).

For each animal, positive nuclei were quantified in area CA1 of the dHPC (dCA1) and the dorsal part of the striatum according to (Franklin & Paxinos, 1997). At least three consecutive serial sections were examined bilaterally, and the number of positive nuclei / mm² was averaged to produce Group mean + SEM and compared to that of home-cage (naive) mice.

2.6. Statistical tests. The behavioral data was analyzed using one- and two-way analysis of variance (ANOVA) for repeated measures with Group as between-subject factor, and Trial as within-subject factors. Within-group comparisons between the percentages of each strategy selection used paired *t*-tests to determine which response type was preferred. χ^2 Analyses were computed in order to determine if any training groups exhibited a significant bias toward one type of strategy. Immunohistochemical data were analyzed using one-way or two-way ANOVAs followed by Fishers Least Significant Difference (LSD) post-hoc tests, when appropriate. A significance level of 0.05 was used for all statistical tests.

3. Results

3.1. Pharmacological inhibition of G9a/GLP activity in the dorsal hippocampus impairs consolidation of long-term spatial memory

We first examined the effect of post-training G9a/GLP blockade in the dHPC on consolidation of spatial LTM. Bilateral infusion of the G9a/GLP inhibitor BIX01294 (*BIX*) or aCSF (*Vehicle*) was administered immediately or 1 hour after the training session consisting of 8 trials followed 24h later, by a probe test in which spatial and cue-guided strategies were in competition (Fig. 1A).

During the acquisition phase, all groups of mice showed a clear improvement in locating the cue-guided PF as indicated by a progressive decrease in escape latencies during the course of learning (Fig. 1B). A two way repeated-measures ANOVA for swim latencies to escape yielded a significant Trial effect ($F_{(7,245)} = 25.15$; $p < 0.0001$), with no significant Group effect ($F_{(1,35)} < 1$; $p = 0.65$), or interaction between Group X Trial ($F_{(7,245)} < 1$; $p = 0.69$), reflecting the fact that the mice had not received any drug up to this point. Therefore, mice were randomly assigned to one of four groups and received either vehicle or BIX treatment immediately or 1hour after the last training trial. Then, a competitive probe test was conducted 24h later in which mice were allowed to freely swim to either the original (spatial) or new (cued) PF location.

First, all vehicle-infused mice showed no strategy preference, regardless of whether they received vehicle infusion immediately or 1h after training (group effect: $F_{(1,13)} = 1.3$; $p = 0.27$; percentage of place strategy: 44.4% and 50%, respectively). However, both vehicle groups spent significantly more time in the half of the pool where the PF was previously located (time spent in spatial area at 0' and 1h : 75.6% and 76.4%, respectively), without significant between group difference ($F_{(1,13)} < 1$; $p = 0.91$). Therefore, we pooled the data for the two vehicle groups together (Fig. 1C_{left} and 1D_{left}).

In mice given immediate post-training infusion of the BIX (BIX-0'; Fig. 1C_{middle}), there was a clear bias towards the use of cue-guided strategy (Spatial: 22.2%; Cued: 77.77%; $p = 0.001$). In contrast, no effect of the drug on mean percentage of spatial *versus* cue response was found when BIX infusion occurred 1h after training ($p = 0.46$; Fig. 1C_{right}). The analysis of the mean percentage of time the animals spent in each half part of the pool confirmed these results (Fig. 1D). Specifically, the BIX-1h group spent significantly more time in the half of the pool containing the spatial PF (70.9%, spatial *versus* cue: $p = 0.018$), similarly to the vehicle one (75.9%; spatial *versus* cue: $p < 0.0001$). In sharp contrast, the exploration of the spatial area was significantly reduced in the BIX-0' group compared to vehicles ($p = 0.0017$) and BIX-1h ($p = 0.018$) due to no such difference in percentage of time exploring the spatial area (50.3%; spatial *versus* cue: $p < 0.82$). An additional analysis of the profiles distribution (Fig. 1E) confirmed that there was a significant drug effect between vehicle and BIX-0' groups ($\chi^2 = 6.075$; $p = 0.013$) with almost all immediate BIX-infused animals displaying a cued profile (BIX: Spatial: 8.3% and Cue: 91.7%; Vehicle: 53.3% and Cue: 46.7%). In contrast, the profile distribution observed in the BIX-1h mice mirrored that of vehicle controls (BIX-1h: Spatial: 60% and Cued: 40%; BIX effect: $\chi^2 = 0.108$; $p = 0.74$). Together, these results suggest that G9a/GLP inhibition in the dHPC results in a significant impairment of spatial LTM, when administrated within a very restricted time-window after the last acquisition trial.

3.2. Pharmacological inhibition of G9a/GLP activity in the dorsal hippocampus has no effect on short-term memory

We next sought to examine whether immediate post-training infusion of BIX interferes with STM during a competition test conducted 1h after training (Fig. 2A). As expected, both Vehicle and BIX groups learned to reach the cue-guided PF efficiently over the 8 training trials (Fig. 2B). In the 1h-competition test, there was no difference between groups in mean

percentage of (cue, place) response ($F_{(1,15)} < 1$; $p = 0.76$; Fig. 2C) or mean percentage of time exploring each half of the pool containing the spatial or cued PF ($F_{(1,15)} = 1.48$; $p = 0.24$; Fig. 2D). Moreover, the profiles of the BIX and Vehicle groups did not significantly differ in the type of strategy used over the three competition trials ($\chi^2 = 0.108$; $p = 0.74$; Fig. 2E). Together, these results indicate that blockade of hippocampal G9a/GLP activity selectively impairs the 24h-retention of spatial LTM but had no effect on STM retention.

3.3. The effects of immediate post-training intra-HPC BIX infusion differ as a function of the training regimen

Then, we examined whether immediate post-training BIX infusion disrupts spatial LTM consolidation in a cohort of mice performing prolonged training regimen (12 training trials; Fig. 3A). During the acquisition phase, both Vehicle and BIX groups learned to locate the cue-guided PF as attested by a progressive decrease in escape latencies over training trials, reaching asymptotic performance at approximately trials 9-10 (Fig. 3B). Two way repeated-measures ANOVA confirmed a significant effect of Trials ($F_{(11,187)} = 27.96$; $p < 0.0001$) but no significant effect of Group ($F_{(1,17)} < 1$; $p = 0.23$) and no significant Group X Trial interaction ($F_{(11,187)} < 1$; $p = 0.83$), reflecting the fact that the mice had not received any drug up to this point.

During the 24h-competition test, Vehicle- and BIX-infused animals showed a slight preference towards the use of spatial strategy to solve the task (vehicle: 62.5% and BIX: 60.6%; Fig. 3C). Indeed, ANOVAs revealed no significant effects of Group for either mean percentage of strategy response ($F_{(1,17)} < 1$; $p = 0.93$) or mean percentage of time the animals spent in each half of the pool ($F_{(1,17)} < 1$; $p = 0.53$; Fig. 3D). The analysis of the profile distribution for each group indicated vehicle mice displayed a bias toward the spatial profile. Even if the BIX mice rather displayed similar rates of spatial and cued strategies, the

distribution of the profiles between the two groups failed to reach significance ($\chi^2 = 2.1$; $p = 0.14$). Overall, these results indicate that deleterious effects of post training BIX infusion on spatial LTM depend on training regimen and/or the performance level reached in acquisition.

3.4. Spatial training differentially regulates H3K9ac and H3K9me2 levels in the dorsal hippocampus and dorsal striatum

There is substantial evidence that dynamic changes of histone H3K9 acetylation and methylation marks are critical players for coordinated activation and repression of genes that are required for the process of LTM formation (Gupta-Agarwal et al., 2012; Gupta et al., 2010; Mahan et al., 2012; Mahan & Ressler, 2012). Therefore, we next investigated the temporal changes in H3K9ac and H3K9me2 levels in the dHPC and the dorsal striatum. To assess this, separate groups of mice were killed before (naïve controls) or immediately, 1h or 24h after the 8-trial acquisition session. Then, the numbers of positive neurons expressing H3K9ac or H3K9me2 were measured in the dorsal CA1 (dCA1) and the dorsomedial (SDM) and dorsolateral (SDL) parts of the striatum using immunohistochemistry.

In the dCA1, the number of positive H3K9ac neurons was significantly increased 1h after training and remained high for up to 24h compared to naïve controls (Fig. 4A). A one-way ANOVA for H3K9ac in the dCA1 area yielded a significant Group effect ($F_{(3,18)} = 16.30$; $p < 0.0001$). Post-hoc analyses confirmed that the trained groups sacrificed at 1h and 24h significantly differed from naïve controls (both $p < 0.001$) and from the group sacrificed immediately after training ($p < 0.001$ and $p < 0.0001$, respectively). In contrast to H3K9ac, no effect of training was observed for H3K9me2 in the dCA1 ($F_{(3,18)} = 0.96$; NS; Fig. 4B).

In the dorsal striatum, one-way ANOVA for H3K9ac revealed main Group effects in both the SDM ($F_{(3,18)} = 15.06$; $p < 0.0001$; Fig. 4C) and the SDL ($F_{(3,18)} = 16.97$; $p < 0.001$; Fig. 4E),

which reflected higher levels of H3K9ac in the trained Group-0' (for SDL only: $p < 0.05$) and Group-1h (SDM and SDL: both $p < 0.001$), compared to naïve animals. We also observed Group-1h was significantly higher than Group-0' (SDM: $p < 0.001$ and SDL: $p < 0.01$) or Group-24h (SDM and SDL: both $p < 0.001$), which otherwise did not differ from each other. The ANOVA for H3K9me2 revealed a significant Group effect in the SDM ($F_{(3,18)} = 5.24$, $p < 0.01$) and the SDL ($F_{(3,18)} = 3.19$, $p = 0.05$), which was due to an early and transient decrease of H3K9me2 in the Group-0' (SDM and SDL: both $p < 0.01$) relative to naïve controls. Post-hoc analyses also indicated that the Group-0' had significantly reduced H3K9me2 compared to other trained groups in the SDM (1h: $p < 0.01$; 24h: $p < 0.05$), but not in the SDL. Together, these results indicate that training produced region-specific changes of H3K9ac and H3K9me2 in the dorsal HPC and the dorsal striatum. In particular, the findings show that training elicited a prolonged increase of H3K9ac in the dCA1 that lasts for up to at least 24h and only a transient peak of H3K9ac in the dorsal striatum whereas H3K9me2 levels are either unchanged (in the dCA1) or transiently reduces (in the striatum) after training.

3.5. G9a/GLP inhibition impairs spatial memory via the deregulation of the balance between H3K9me2 and H3K9ac levels in the dorsal hippocampus.

Next, we tested the hypothesis that the impairing effects of the BIX inhibitor on spatial LTM is the result of a dysregulation of the balance between H3K9Ac/H3K9me2 in the HPC and the striatum. To the end, four groups of mice received intra-HPC infusion of either the BIX or vehicle immediately after training and were killed 1h or 24h later. We then analysed the effects of local BIX infusion on H3K9ac and H3K9me2 in the dCA1 and the dorsal striatum using immunohistochemistry (Fig. 5).

In the dCA1, a 2-way ANOVA for H3K9ac data revealed main effects of Group ($F_{(1,13)} = 14.29$; $P = 0.023$), of Time ($F_{(1,13)} = 8.86$; $P = 0.01$) and a significant Group X Time interaction

($F_{(1,13)} = 14.85$; $P = 0.002$). For H3K9me2 in the dCA1, there was a main effect of group ($F_{(1,13)} = 7.25$; $P = 0.018$), of time ($F_{(1,13)} = 8.47$; $P = 0.012$) but no interaction ($F_{(1,13)} = 3.52$; $P = 0.083$). Relative to vehicle controls, the BIX-1h mice displayed significantly increased levels of H3K9Ac ($P < 0.001$; Fig. 5A and 5C) as opposed to significantly reduced H3K9me2 levels ($-61.4 \pm 7\%$; $P < 0.01$; Fig. 5B and 5D). In contrast, no group difference was observed for either H3K9ac or H3K9me2 at 24h post-training (*versus* vehicle: both $P > 0.5$; *versus* 1h: $P < 0.001$ and $P < 0.01$, respectively).

In the dorsal striatum, statistical analyses detected no effect of group for H3K9me2 or for H3K9ac neither in the SDM nor in the SDL (all $F < 1.5$; all $P > 0.1$) (data not shown).

These results therefore suggested that reduced G9a activity, leading to altered balance between H3K9ac and H3K9me2, selectively in the dCA1 during the early consolidation phase is sufficient to disrupt spatial LTM to the benefit of cued memory.

4. Discussion

In the present study, we used a dual-solution version of the water maze to examine the role of the H3K9-specific histone methyltransferase (HMT) G9a/G9a-like protein (GLP) activity in LTM consolidation of spatial information and dynamic interaction between HPC-dependent spatial/place and dorsal striatum-dependent cued-response memory systems. First, our behavioural experiments indicate that intra-CA1 infusion of the G9a/GLP HMT inhibitor BIX01294 administrated immediately after a moderate training session (8-trial session) interferes with the selection of the HPC-dependent spatial/place strategy and leads to maintain a predominant use of the striatum-based cue strategy as assessed during the competition test 24h later. However, the failure to switch to the spatial strategy caused by BIX was not observed when the drug was administered 1 hour after training or when the mice were given four additional training trials (12-trial session). Second, we showed that mice submitted to the 8-trial training regimen display no change in H3K9me2 in the dCA1 and only an immediate, transient decrease of H3K9me2 in the dorsal striatum, compared to naïve controls. In contrast, training elicited an increase of H3K9ac level in the dCA1 and the dorsal striatum that persisted up to 24h in the dCA1 but returned near to basal level in the dorsal striatum. Third, our imaging data indicated that post-training BIX infusion into the dCA1 may impair spatial LTM formation via transient alteration of the balance between H3K9me2 (reduced) and H3K9ac (increased) that lasts only few hours after learning. Together, these data suggest that G9a/GLP complex activity, along with the balance between histone H3K9 acetylation and methylation, is involved in the epigenetic gene control mechanisms underlying LTM formation.

Previous investigations using dual-solution water maze tasks that can be solved with both HPC-dependent spatial/place and striatum-dependent cue-guided navigational strategies have shown that animals initially adopt a cued/response strategy early in training but then, with learning progression or when the context pre-exposure is sufficient, they switch toward predominant use of spatial/place strategy (Martel et al., 2007; Martel, Millard, Jaffard, & Guillou, 2006; Nicolle, Prescott, & Bizon, 2003; Sung et al., 2008; Tunur, Dohanich, & Schrader, 2010). In this regard, we previously showed that pharmacological elevation of histone acetylation in the dorsal HPC immediately post-learning operates as a molecular switch between the HPC- and dorsal striatum-dependent memory systems, biasing mice towards predominant use of spatial navigational strategies under training condition that normally promotes the use of cue strategy in vehicle controls (Dagnas et al., 2013). Importantly, recent evidence clearly demonstrated the importance of transcriptionally repressive histone H3K9me2, catalyzed by the HMT G9a/GLP complex, in coordinating cellular and molecular connectivity between the dorsal HPC and other brain regions during fear LTM consolidation and storage (Gupta-Agarwal et al., 2012). Specifically, inhibition of the G9a-mediated H3K9me2 with the selective inhibitor of G9a activity BIX01294 improved or impaired contextual fear LTM depending on whether the drug was infused into the HPC or the entorhinal cortex, indicating that the same histone modification can exert brain region-specific outcomes on memory processes. An essential role for the HMT G9a and its downstream H3K9me2 in fear memory LTM has also been reported in studies using mice with postnatal conditional knockout of G9a/GLP in the forebrain (Maze et al., 2010). The above findings prompted us to examine whether pharmacological disruption of G9a-mediated H3K9me2 in the dorsal HPC, using the selective inhibitor of G9a/GLP activity BIX01294, immediately after learning affects LTM of spatial/place information in a water maze competition task that can be solved with both HPC-dependent spatial/place and dorsal striatal-

dependent cue memory systems. The main finding in our study was that intra-CA1 inhibition of the G9a-mediated H3K9me2 immediately after learning elicits a functional imbalance between dHPC and dorsal striatum, prompting the predominant use of striatum-dependent cue-guided search strategies during the competition test assessed 24h after learning. Specifically, vehicle-infused mice subjected to 8-trial acquisition did not discriminate 24 h later between the place and cue strategies, independently of the time interval (1h or 24h) interposed between the acquisition and the test session. In contrast, the BIX-infused mice were able to successfully use both place and cued strategies on the 1h-probe test but exhibited significantly greater use of a cue/response strategy on the 24h-probe test. Together, the present data indicate that pharmacological inhibition of hippocampal G9a/GLP complex during the initial consolidation window impaired HPC-dependent spatial LTM when mice were tested 24h after learning, while leaving protein synthesis-independent spatial STM unaffected. These results strongly suggest a critical role for the G9a/GLP complex activity as part of a molecular cascade of early transcription events that is required in the dorsal HPC for proper long-term consolidation of spatial memory. Our data are consistent with previous findings wherein the pharmacological inhibition of G9a/GLP activity in the HPC impairs LTM formation of contextual fear conditioning, accelerates extinction and prevents the induction and maintenance of late long-term potentiation (L-LTP) at the schaffer collateral-CA1 synapses, a cellular correlate of HPC-dependent LTM formation (Gupta-Agarwal et al., 2012). On the other hand, slight inhibition of G9a/GLP complex activity using lower inhibitor concentrations reverses long-term depression (LTD), reinforces LTP maintenance and promotes synaptic tagging/capture in hippocampal CA1 pyramidal neurons (Sharma, Razali, & Sajikumar, 2017). Interestingly, the authors reported that such effects of G9a/GLP inhibition on LTP maintenance occurred within a limited time-frame of 30min before and after the induction of early LTP, which is consistent with our data reporting an impairing

effect of the BIX01294 inhibitor on spatial LTM when administrated immediately, but not 60min, after learning. According to the above findings, the same authors have point out a key role for G9a/GLP complex, as an epigenetic bidirectional regulator of the switch from short- to long-term synaptic plasticity and memory, through transcriptional and/or translational control of specific set of plasticity-related products, such as brain-derived-neurotropic factor (BDNF), protein kinase M ζ and activity-related cytoskeletal (Arc) genes (Pang, Sharma, & Sajikumar, 2019).

One of the multiple factors that can influence dynamic interaction between the dorsal HPC- and dorsal striatum-dependent memory systems to direct behavior is the amount of training that animals have received (Packard & Goodman, 2013). Numerous studies including ours showed that very early learning is controlled by the striatum-dependent memory whereas the use of HPC-dependent place/spatial memory comes to dominate on the condition that training or pre-exposure to the spatial environment is sufficient (Martel et al., 2007; Martel et al., 2006; Nicolle et al., 2003; Sung et al., 2008; Tunur et al., 2010). In line with these findings, the current data showed that vehicle mice that had received 12 training trials spent more time searching in half of the pool containing the spatial PF and relied predominantly on the place strategy during the 24h-probe test, compared with those that underwent 8 training trials. Moreover, intra-CA1 infusion of the BIX immediately after the last 12th trial had no significant effect on spatial LTM retention, suggesting that after the 12-trials training session, once mice had reached asymptotic level of performance, consolidation of previously acquired information is already stabilized and no longer susceptible to disruption by G9a/GLP inhibitors.

Since the transcriptionally repressive H3K9me2 is mutually exclusive to permissive H3K9ac, we investigated whether regional and temporal patterns of H3K9me2 and H3K9Ac in the dHPC and the dorsal striatum correlate with strategy engagement in vehicle- and BIX-infused

mice. Our results indicate that vehicle mice submitted to 8 acquisition trials exhibited significant increases in H3K9ac at 1h and 24h post-training in area CA1 and a transient peak of H3K9ac at 1h in the dorsal striatum, compared with naïve controls. Previously, H3K9me2 was found to be persistently altered in the HPC following associative contextual fear conditioning (Gupta et al., 2010), which contrasts with our results showing no effect of training on H3K9me2 in area CA1 and an early, transient decrease of H3K9me2 in the dorsal striatum. Previous studies reported that local inhibition of G9a/GLP activity 1h prior to conditioning disrupts the dynamic balance between transcription-repressive H3K9me2 and transcription-permissive H3K9ac and H3K4me3 marks across multiple brain regions recruited for LTM formation (Gupta-Agarwal et al., 2012). We also observed that G9a/GLP inhibition in dorsal CA1 immediately after learning resulted in the down-regulation of H3K9me2 and further elevation of H3K9ac in area CA1 at 1h post-training, compared with vehicle condition. Importantly, the effects of the BIX on the balance between histone acetylation and methylation appears to be restricted to a limited time-window as we found no difference between BIX- and vehicle-infused animals sacrificed 24h after training. In addition, intra-CA1 administration of BIX did not alter the levels of H3K9ac and H3K9me2 in the dorsal striatum. Overall, our results indicate that mice with disrupted G9a/GLP activity in area CA1 display spatial LTM impairment, which was accompanied by altered balance between H3K9ac (increased) and H3K9me2 (decreased) during initial consolidation period. Therefore, G9a/GLP activity in area CA1 may be of critical importance for the fine-tuning of transcriptional gene activation and silencing necessary for HPC-dependent LTM formation. Accordingly, an essential role for G9a/GLP activity has recently emerged as a bidirectional regulator of synaptic plasticity, through its transcriptional corepressor (e.g. *Zif268*, *BDNF exon IV*, *DNMT3a* genes) or coactivator (e.g. non-permissive *COMT* gene) functions (Bittencourt et al., 2012; Gupta-Agarwal et al., 2012) for review, (Pang et al., 2019)). Several studies have

suggested that H3K9 lysine methyltransferase promoters (for example, G9a) dynamically interact with HDACs to regulate functional interplay between histones H3K9ac and H3K9me2 within the genome (Kennedy et al., 2013; Maze et al., 2010). For instance, Gupta and colleagues (2010) proposed that one mechanism by which HDAC inhibition produces memory enhancement may be through the negative regulation of hippocampal G9a-mediated H3K9me2 (Gupta et al., 2010). Together, the present data provide evidence that proper regulation of G9a-mediated H3K9me2 activity occurring in area CA1 during initial consolidation window is necessary for long-term consolidation processes of spatial memory. During recent years, growing body of evidence has emerged to suggest sex differences in regionally specific recruitment of histone PTMs and histones modifying enzymes involved in regulating transcription of genes important for learning and/or memory (Keiser & Wood, 2019). Interestingly, a recent study revealed that different behavioral experiences produced sex-specific differences of global histone H3K9 acetylation and methylation patterns, both within and across the dorsal HPC and frontal cortex (Sobolewski, Singh, Schneider, & Cory-Slechta, 2018). Furthermore, higher levels of both HDAC activity and/or DNA methyltransferase 1 content have been reported in the HPC and cortices of females compared to males, indicators of increased transcriptional activity repression (Elsner, Cechinel, de Meireles, Bertoldi, & Siqueira, 2018; Tsai, Grant, & Rissman, 2009). Since our data were obtained from adult male mice, it is important for future studies to precisely determine sex-dependent difference to the behavioral effect of local intra-HPC G9a/GLP inhibition in female mice.

Overall, the data indicate that deregulation of the functional interplay between H3K9ac (increased) and H3K9me2 (decreased) during initial consolidation window in area CA1 could be causing the memory-disrupting effects of BIX. Although molecular details on how G9a-

mediated H3K9me2 and other chromatin-modifying enzymes such as HDAC interact to regulate memory formation remain to be elucidated, these results may have important therapeutic implications in view of the efforts to design new drugs to target cognitive disorders resulting from normal and pathological aging.

Data availability statement.

Data is available from the authors upon reasonable request.

References

- Benevento, M., van de Molengraft, M., van Westen, R., van Bokhoven, H., & Kasri, N. N. (2015). The role of chromatin repressive marks in cognition and disease: A focus on the repressive complex GLP/G9a. *Neurobiol Learn Mem*, 124, 88-96. doi:10.1016/j.nlm.2015.06.013
- Bittencourt, D., Wu, D. Y., Jeong, K. W., Gerke, D. S., Herviou, L., Ianculescu, I., . . . Stallcup, M. R. (2012). G9a functions as a molecular scaffold for assembly of transcriptional coactivators on a subset of glucocorticoid receptor target genes. *Proc Natl Acad Sci U S A*, 109(48), 19673-19678. doi:10.1073/pnas.1211803109
- Bousiges, O., Vasconcelos, A. P., Neidl, R., Cosquer, B., Herbeaux, K., Panteleeva, I., . . . Boutillier, A. L. (2010). Spatial memory consolidation is associated with induction of several lysine-acetyltransferase (histone acetyltransferase) expression levels and H2B/H4 acetylation-dependent transcriptional events in the rat hippocampus. *Neuropsychopharmacology*, 35(13), 2521-2537. doi:npp2010117 [pii] 10.1038/npp.2010.117
- Castellano, J. F., Fletcher, B. R., Kelley-Bell, B., Kim, D. H., Gallagher, M., & Rapp, P. R. (2012). Age-related memory impairment is associated with disrupted multivariate epigenetic coordination in the hippocampus. *PLoS One*, 7(3), e33249. doi:10.1371/journal.pone.0033249 PONE-D-12-03985 [pii]
- Castellano, J. F., Fletcher, B. R., Patzke, H., Long, J. M., Sewal, A., Kim, D. H., . . . Rapp, P. R. (2014). Reassessing the effects of histone deacetylase inhibitors on hippocampal memory and cognitive aging. *Hippocampus*, 24(8), 1006-1016. doi:10.1002/hipo.22286
- Dagnas, M., Guillou, J. L., Prevot, T., & Mons, N. (2013). HDAC inhibition facilitates the switch between memory systems in young but not aged mice. *J Neurosci*, 33(5), 1954-1963. doi:10.1523/JNEUROSCI.3453-12.2013
- Dagnas, M., Micheau, J., Decorte, L., Beracochea, D., & Mons, N. (2015). Post-training, intrahippocampal HDAC inhibition differentially impacts neural circuits underlying spatial memory in adult and aged mice. *Hippocampus*, 25(7), 827-837. doi:10.1002/hipo.22406
- Dagnas, M., & Mons, N. (2013). Region- and age-specific patterns of histone acetylation related to spatial and cued learning in the water maze. *Hippocampus*. doi:10.1002/hipo.22116
- Elsner, V. R., Cechinel, L. R., de Meireles, L. C. F., Bertoldi, K., & Siqueira, I. R. (2018). Epigenetic marks are modulated by gender and time of the day in the hippocampi of adolescent rats: A preliminary study. *Neural Regen Res*, 13(12), 2160-2163. doi:10.4103/1673-5374.241467
- Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L. H. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature*, 447(7141), 178-182. doi:nature05772 [pii] 10.1038/nature05772
- Franklin, K. B. J., & Paxinos, G. (1997). *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, San Diego, C.
- Gasser, J., Pereira de Vasconcelos, A., Cosquer, B., Boutillier, A. L., & Cassel, J. C. (2020). Shifting between response and place strategies in maze navigation: Effects of training, cue availability and functional inactivation of striatum or hippocampus in rats. *Neurobiol Learn Mem*, 167, 107131. doi:10.1016/j.nlm.2019.107131
- Gupta-Agarwal, S., Franklin, A. V., Deramus, T., Wheelock, M., Davis, R. L., McMahon, L. L., & Lubin, F. D. (2012). G9a/GLP histone lysine dimethyltransferase complex

- activity in the hippocampus and the entorhinal cortex is required for gene activation and silencing during memory consolidation. *J Neurosci*, 32(16), 5440-5453. doi:10.1523/JNEUROSCI.0147-12.2012
- Gupta, S., Kim, S. Y., Artis, S., Molfese, D. L., Schumacher, A., Sweatt, J. D., . . . Lubin, F. D. (2010). Histone methylation regulates memory formation. *J Neurosci*, 30(10), 3589-3599. doi:10.1523/JNEUROSCI.3732-09.2010
- Keiser, A. A., & Wood, M. A. (2019). Examining the contribution of histone modification to sex differences in learning and memory. *Learn Mem*, 26(9), 318-331. doi:10.1101/lm.048850.118
- Kennedy, P. J., Feng, J., Robison, A. J., Maze, I., Badimon, A., Mouzon, E., . . . Nestler, E. J. (2013). Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci*, 16(4), 434-440. doi:10.1038/nn.3354
- Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. *J Biol Chem*, 279(39), 40545-40559. doi:10.1074/jbc.M402229200 M402229200 [pii]
- Mahan, A. L., Mou, L., Shah, N., Hu, J. H., Worley, P. F., & Ressler, K. J. (2012). Epigenetic modulation of Homer1a transcription regulation in amygdala and hippocampus with pavlovian fear conditioning. *J Neurosci*, 32(13), 4651-4659. doi:10.1523/JNEUROSCI.3308-11.2012
- Mahan, A. L., & Ressler, K. J. (2012). Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends Neurosci*, 35(1), 24-35. doi:10.1016/j.tins.2011.06.007
- Martel, G., Blanchard, J., Mons, N., Gastambide, F., Micheau, J., & Guillou, J. L. (2007). Dynamic interplays between memory systems depend on practice: the hippocampus is not always the first to provide solution. *Neuroscience*, 150(4), 743-753.
- Martel, G., Millard, A., Jaffard, R., & Guillou, J. L. (2006). Stimulation of hippocampal adenylyl cyclase activity dissociates memory consolidation processes for response and place learning. *Learn Mem*, 13(3), 342-348. doi:13/3/342 [pii] 10.1101/lm.149506
- Maze, I., Covington, H. E., 3rd, Dietz, D. M., LaPlant, Q., Renthal, W., Russo, S. J., . . . Nestler, E. J. (2010). Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science*, 327(5962), 213-216. doi:10.1126/science.1179438
- Nicolle, M. M., Prescott, S., & Bizon, J. L. (2003). Emergence of a cue strategy preference on the water maze task in aged C57B6 x SJL F1 hybrid mice. *Learn Mem*, 10(6), 520-524. doi:10.1101/lm.64803 10/6/520 [pii]
- Packard, M. G., & Goodman, J. (2013). Factors that influence the relative use of multiple memory systems. *Hippocampus*, 23(11), 1044-1052. doi:10.1002/hipo.22178
- Pang, K. K. L., Sharma, M., & Sajikumar, S. (2019). Epigenetics and memory: Emerging role of histone lysine methyltransferase G9a/GLP complex as bidirectional regulator of synaptic plasticity. *Neurobiol Learn Mem*, 159, 1-5. doi:10.1016/j.nlm.2019.01.013
- Peixoto, L., & Abel, T. (2012). The Role of Histone Acetylation in Memory Formation and Cognitive Impairments. *Neuropsychopharmacology*. doi:10.1038/npp.2012.86 npp201286 [pii]
- Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R. C., . . . Fischer, A. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science*, 328(5979), 753-756. doi:328/5979/753 [pii]10.1126/science.1186088
- Rice, J. P., Wallace, D. G., & Hamilton, D. A. (2015). Lesions of the hippocampus or dorsolateral striatum disrupt distinct aspects of spatial navigation strategies based on

- proximal and distal information in a cued variant of the Morris water task. *Behav Brain Res*, 289, 105-117. doi:10.1016/j.bbr.2015.04.026
- Schaefer, A., Sampath, S. C., Intrator, A., Min, A., Gertler, T. S., Surmeier, D. J., . . . Greengard, P. (2009). Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex. *Neuron*, 64(5), 678-691. doi:10.1016/j.neuron.2009.11.019
- Sharma, M., Dierkes, T., & Sajikumar, S. (2017). Epigenetic regulation by G9a/GLP complex ameliorates amyloid-beta 1-42 induced deficits in long-term plasticity and synaptic tagging/capture in hippocampal pyramidal neurons. *Aging Cell*, 16(5), 1062-1072. doi:10.1111/accel.12634
- Sharma, M., Razali, N. B., & Sajikumar, S. (2017). Inhibition of G9a/GLP Complex Promotes Long-Term Potentiation and Synaptic Tagging/Capture in Hippocampal CA1 Pyramidal Neurons. *Cereb Cortex*, 27(6), 3161-3171. doi:10.1093/cercor/bhw170
- Sobolewski, M., Singh, G., Schneider, J. S., & Cory-Slechta, D. A. (2018). Different Behavioral Experiences Produce Distinctive Parallel Changes in, and Correlate With, Frontal Cortex and Hippocampal Global Post-translational Histone Levels. *Front Integr Neurosci*, 12, 29. doi:10.3389/fnint.2018.00029
- Stilling, R. M., & Fischer, A. (2011). The role of histone acetylation in age-associated memory impairment and Alzheimer's disease. *Neurobiol Learn Mem*, 96(1), 19-26. doi:S1074-7427(11)00064-5 [pii] 10.1016/j.nlm.2011.04.002
- Sung, J. Y., Goo, J. S., Lee, D. E., Jin, D. Q., Bizon, J. L., Gallagher, M., & Han, J. S. (2008). Learning strategy selection in the water maze and hippocampal CREB phosphorylation differ in two inbred strains of mice. *Learn Mem*, 15(4), 183-188. doi:15/4/183 [pii] 10.1101/lm.783108
- Sweatt, J. D. (2009). Experience-dependent epigenetic modifications in the central nervous system. *Biol Psychiatry*, 65(3), 191-197. doi:S0006-3223(08)01089-5 [pii] 10.1016/j.biopsych.2008.09.002
- Tsai, H. W., Grant, P. A., & Rissman, E. F. (2009). Sex differences in histone modifications in the neonatal mouse brain. *Epigenetics*, 4(1), 47-53. doi:10.4161/epi.4.1.7288
- Tunur, T., Dohanich, G. P., & Schrader, L. A. (2010). Pre-exposure to context affects learning strategy selection in mice. *Learn Mem*, 17(7), 328-331. doi:17/7/328 [pii] 10.1101/lm.1818410
- Vecsey, C. G., Hawk, J. D., Lattal, K. M., Stein, J. M., Fabian, S. A., Attner, M. A., . . . Wood, M. A. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. *J Neurosci*, 27(23), 6128-6140. doi:27/23/6128 [pii] 10.1523/JNEUROSCI.0296-07.2007

Figure legends

Figure 1. Post-training intra-CA1 infusion of the G9a/GLP inhibitor BIX-01294 impairs long-term consolidation of spatial information when administrated immediately but not 1h after training. **A.** Schematic representation of the experimental procedure. During the 8-trial training session, young mice learned to search for a submerged cue-marked platform. Immediately or 1h after training, they received bilateral infusion of BIX (BIX-0': N=12 and BIX-1H: N=10) or aCSF (pooled Vehicle-0' and Vehicle-1H: N= 15) into the dorsal CA1 region (arrows). During the competition test performed 24h later, mice had to choose between a submerged platform located in the same position as during the training session (place strategy) and the cue-marked platform located in the opposite quadrant of the pool (cue strategy). For detailed explanations, see Material and Methods. **B.** Learning curves for Vehicle, BIX-0' and BIX-1H groups during the acquisition session. Data are expressed as mean latency (sec) to find the platform over the 8 consecutive trials. **C.** Mean percentage (+SEM) of (cue, place) strategy selection during the 24 h completion test for Vehicle, BIX-0' and BIX-1H groups. **D.** Mean percentage (+SEM) of time spent by Vehicle, BIX-0' and BIX-1H groups in each half of the pool during the 24h-probe test. **E.** Individual strategy response as expressed as mean percentage of mice that rely on a place or cue strategy during the probe test for each group. * $p < 0.05$, *** $p < 0.001$: cue vs. place strategy; $^{\$} p < 0.05$, $^{\$\$} p < 0.01$ compared with BIX-0' group.

Figure 2. Intra-hippocampal BIX infusion immediately post-training does not affect short-term spatial memory retention. **A.** Experimental design. Mice received bilateral intra-CA1 infusion of BIX (BIX: N= 10) or aCSF (Vehicle: N=7) immediately after training. STM performance was measured 1h later during a competition test consisting of 3 trials with a submerged (place) platform located in the original quadrant and the cue-marked platform moved to the diagonally opposite quadrant. **B.** Learning curves for vehicle and BIX-0' groups expressed as mean latency (sec) to find the platform during the training session. **C-D.** Mean percentage (+SEM) of (cue, place) strategy selection (*C*) and of time spent in each half of the pool (*D*) in vehicle and BIX-0' groups during the 1h-probe test. **E.** Individual strategy response for Vehicle and BIX groups as expressed as mean percentage of mice that rely on a place or cue strategy during the probe test for each group. * $p < 0.05$, *** $p < 0.001$: cue vs. place strategy.

Figure 3. The effect of intra-hippocampal BIX infusion on spatial LTM retention depends on the intensity of training regimen. **A.** Experimental design. Mice were given an acquisition session consisting of 12 trials and, immediately after, they received intra-CA1 infusion of BIX (BIX: N=11) or aCSF (Vehicle: N=8). LTM retention was assessed 24h later during the probe test consisting of 3 trials with a submerged platform placed in the original quadrant (place strategy) and the cue-marked platform moved to the diagonally opposite quadrant (cue strategy). **B.** Learning curves for vehicle and BIX-0' mice expressed as mean escape latency (sec) to locate the platform over the 12 consecutive trials during the acquisition session. **C-D.** Mean percentage (+SEM) of (cue, place) strategy selection (*C*) and of time spent in each half of the pool (*D*) for Vehicle and BIX groups during the 24h-probe test. **E.** Individual strategy response as expressed as mean percentage of mice that rely on a place or a cue strategy for each group during the probe test. $**p < 0.01$, $***p < 0.001$: cue vs. place strategy.

Figure 4. Training induces distinct temporal patterns of H3K9 acetylation and methylation in the dorsal hippocampus and the dorsal striatum. Trained mice were sacrificed at three time-points after the 8-trial training session (Group-0H: N=7; Group-1H: N= 7; Group-24H: N= 4) and compared with Naïve controls (Naïve: N=4). Quantification of immunopositive nuclei for H3K9ac (*left*) and H3K9me2 (*right*) in the dCA1 (**A-B**), the dorso-medial (SDM; **C-D**) and dorso-lateral (SDL; **E-F**) parts of the striatum. Data are represented as mean (+SEM) number of immunoreactive nuclei per mm². $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different from Naïve. $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$ \$ \$}p < 0.001$ significantly different from other trained group.

Figure 5. Training elicits significant increase of H3K9ac and no change of H3K9me² in the dorsal CA1. Representative immunostainings for H3K9ac (left) and H3K9me2 (right) in area CA1 of the dorsal hippocampus for Naïve controls and trained mice sacrificed immediately, 1H or 24H after the 8-trial training session. Scale bar: 25 μ m

Figure 6. Training elicits a transient peak of H3K9ac and an early decrease of H3K9me² in the dorsal striatum. Representative immunostainings for H3K9ac (left) and H3K9me2 (right) in the dorsal striatum for Naïve controls and trained mice sacrificed immediately, 1H or 24H after the 8-trial training session. Scale bar: 25 μ m

Figure 7. Post-training inhibition of G9a/GLP activity affects the balance between H3K9ac and H3K9me2 in the dCA1. Mice received intra-CA1 infusion of BIX or vehicle immediately after the 8-trial training session and were killed 1h or 24h later. **(A-B)** Mean (+ SEM) fold changes of acetyl-H3K9 (A) and H3K9me2 (B) in the dorsal CA1 region of BIX-infused mice (1H-BIX: N=4; 24H-BIX: N=5) relative to respective Vehicles (1H: N=3; 24H: N=5). **(C-D)** Representative H3K9ac (C) and H3K9me2 (D) immunostainings in the dorsal CA1 for vehicle (*left*) and BIX (*right*) mice sacrificed 1h after training. Scale bar: 100 μ m. The *asterisk* indicates the infusion *cannula* location. $**p < 0.01$ and $***p < 0.001$: compared with 1H-vehicle. $^{**}p < 0.01$ and $^{***}p < 0.001$: compared with 24H-BIX.

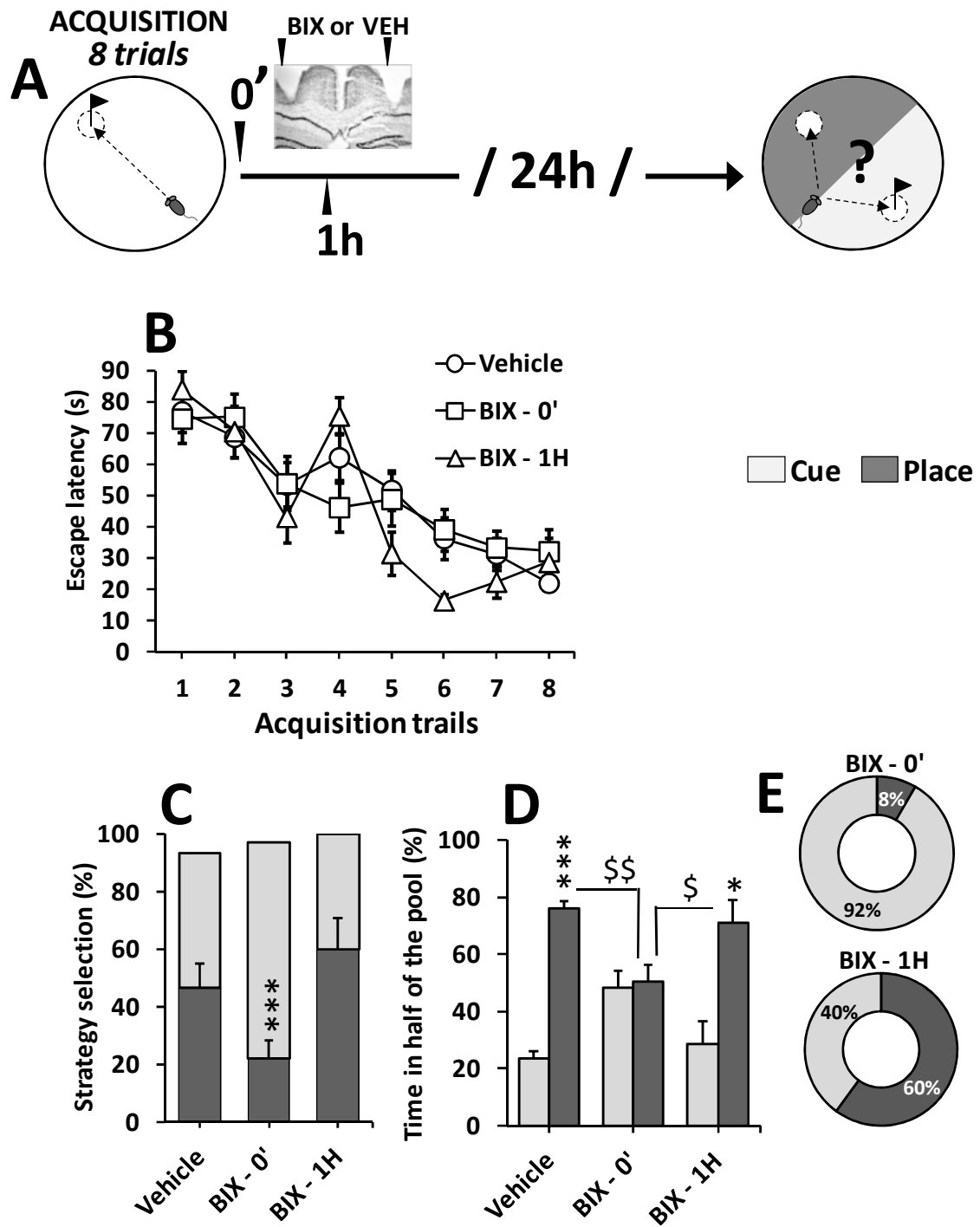


FIGURE 1

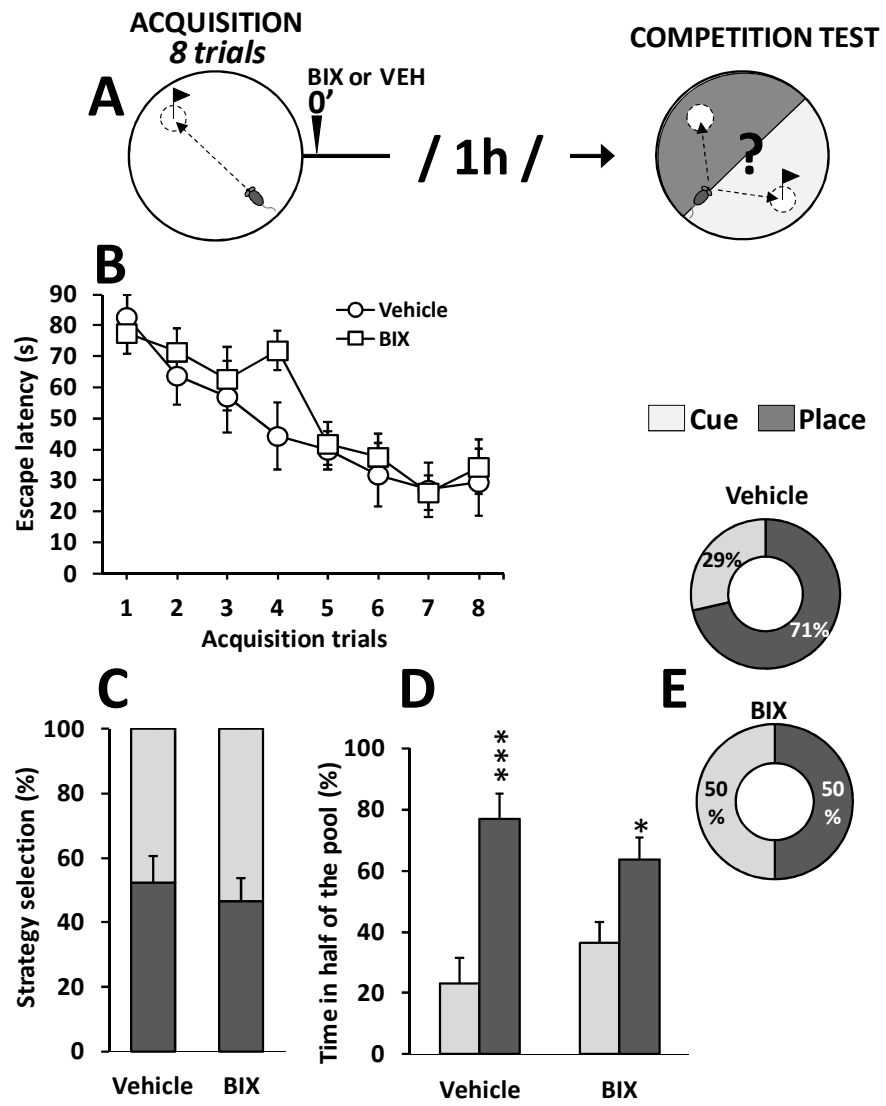


FIGURE 2

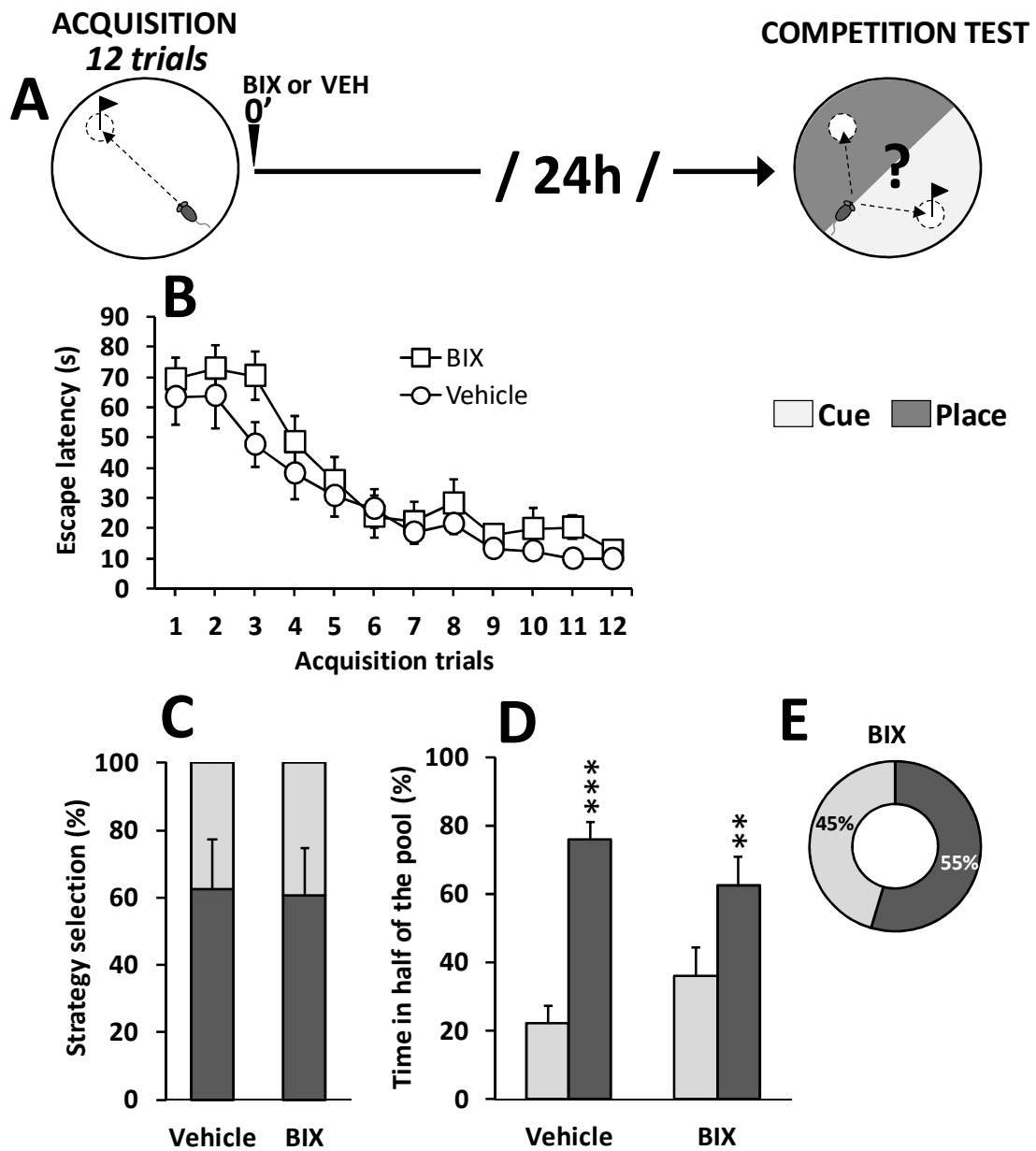


FIGURE 3

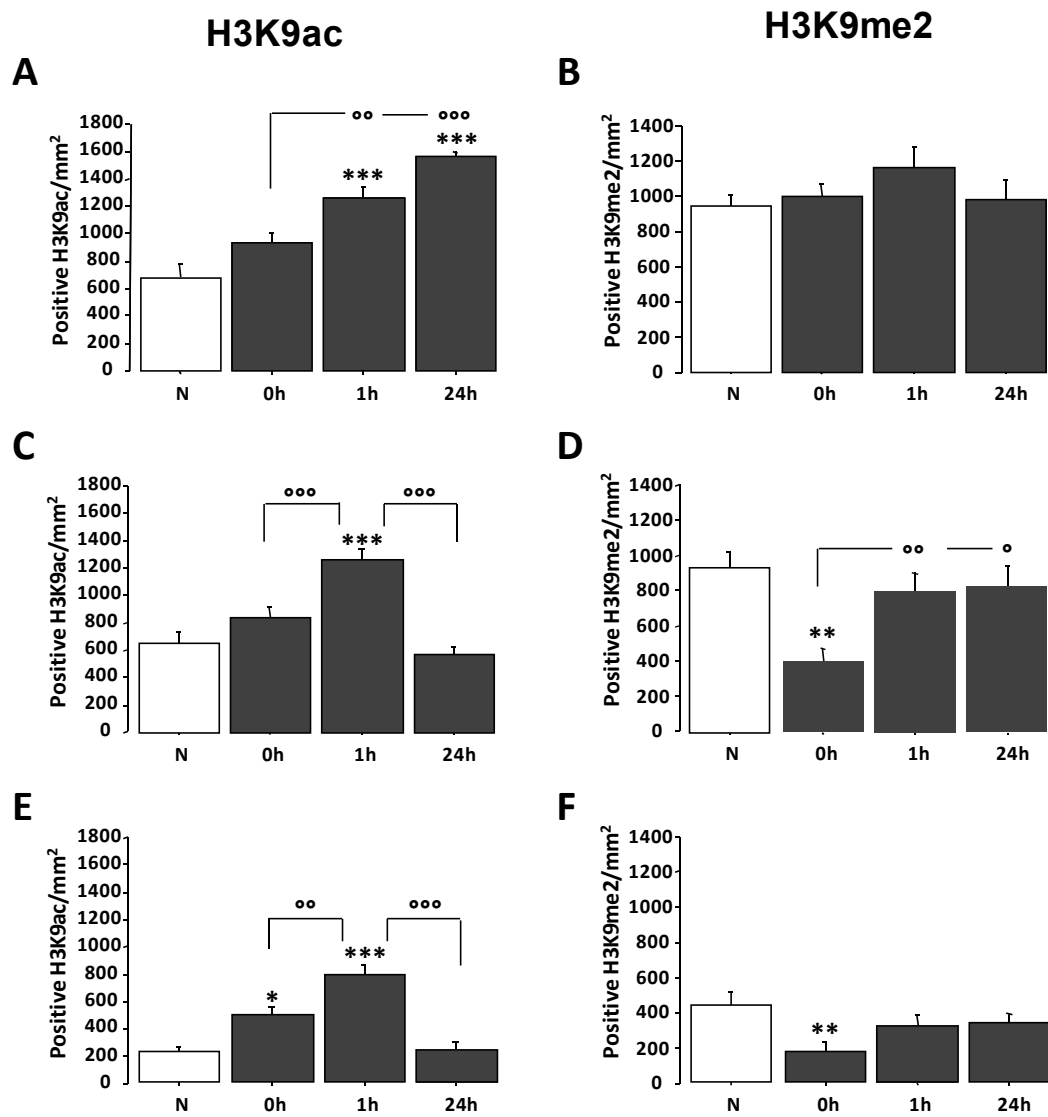


FIGURE 4

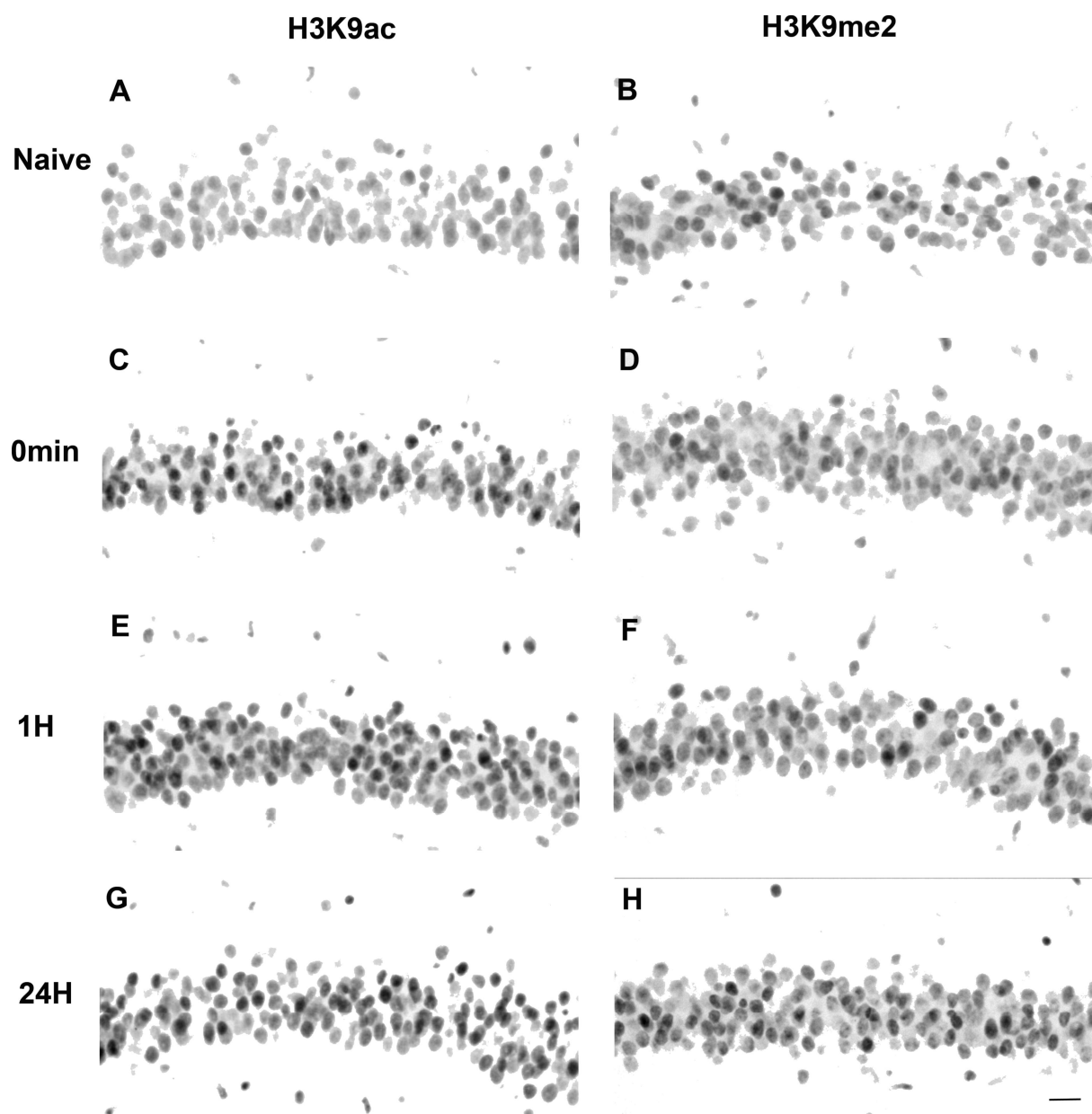


FIGURE 5

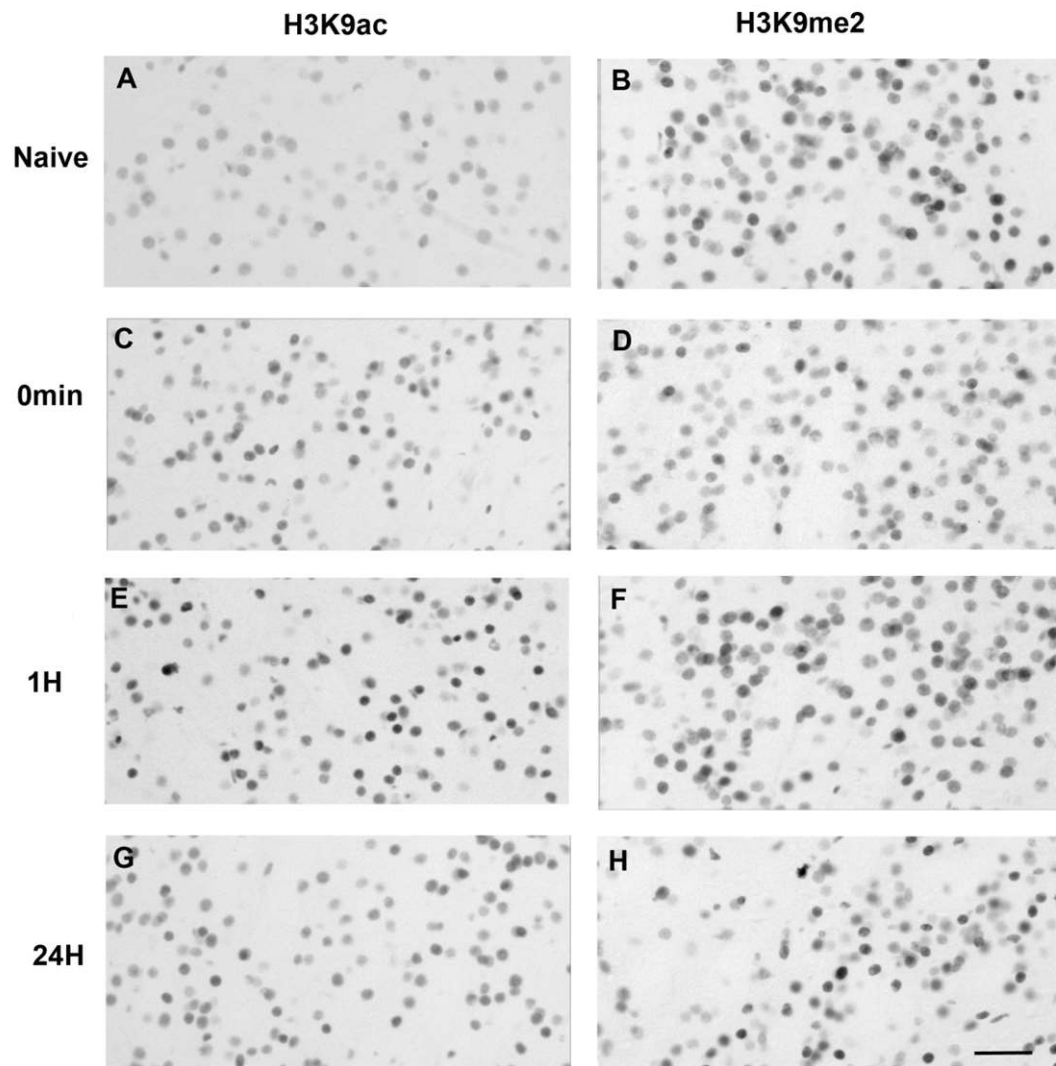


FIGURE 6

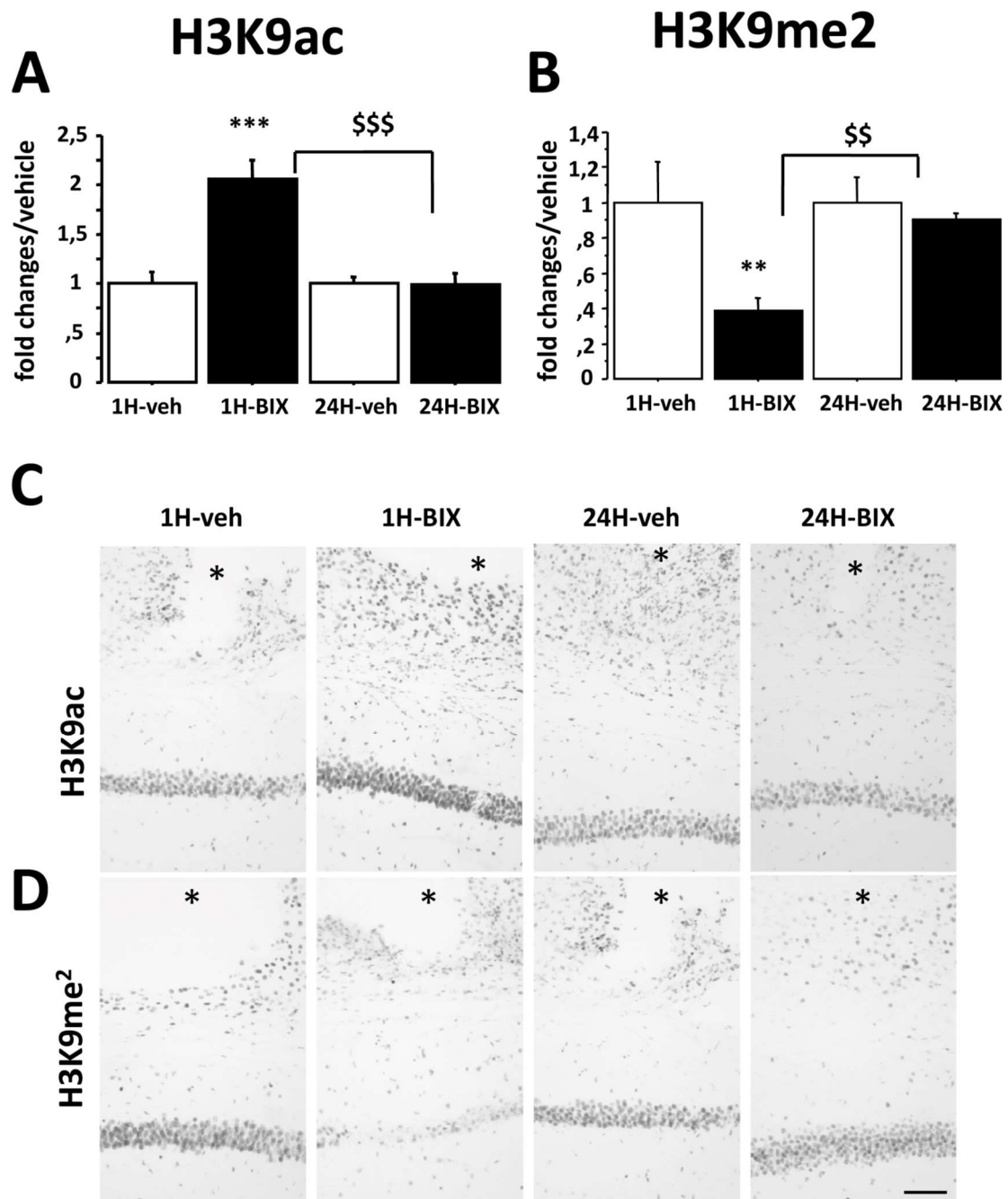


FIGURE 7

Nom du document : Kritter, Lassalle, Guillou, Mons-revised.16janvier.docx
Répertoire : C:\Users\mons\Desktop\Publi-Kyrian\Neurobiol Learn Mem
Modèle : C:\Users\mons\AppData\Roaming\Microsoft\Templates\Normal.dotm
Titre :
Sujet :
Auteur : mons
Mots clés :
Commentaires :
Date de création : 22/11/2021 10:06:00
N° de révision : 7
Dernier enregist. le : 22/11/2021 10:27:00
Dernier enregistrement par : mons
Temps total d'édition : 6 Minutes
Dernière impression sur : 22/11/2021 10:29:00
Tel qu'à la dernière impression
Nombre de pages : 38
Nombre de mots : 8 587 (approx.)
Nombre de caractères : 47 234 (approx.)