Memory and plasticity impairment after binge drinking in adolescent rat hippocampus: GluN2A/GluN2B NMDA receptor subunits imbalance through HDAC2

Ichrak Drissi | Chloé Deschamps | Grégory Fouquet | Rachel Alary | Stéphane Peineau | Philippe Gosset | Harold Sueur | Ingrid Marcq | Véronique Debuysscher | Mickael Naassila | Catherine Vilpoux | Olivier Pierrefiche

UPJV, INSERM UMR 1247 GRAP, Groupe de Recherche sur l’Alcool et les Pharmacodépendances, Centre Universitaire de Recherche en Santé (CURS), Amiens Cedex 1, France

Correspondence
Olivier Pierrefiche, UPJV, INSERM UMR 1247 GRAP, Groupe de Recherche sur l’Alcool et les Pharmacodépendances, Centre Universitaire de Recherche en Santé (CURS), Chemin du Thil, CHU-Amiens Sud, 80025 Amiens, France. Email: olivier.pierrefiche@u-picardie.fr

Funding information
Région Hauts-de-France; INSERM

Abstract
Ethanol (EtOH) induces cognitive impairment through modulation of synaptic plasticity notably in the hippocampus. The cellular mechanism(s) of these EtOH effects may range from synaptic signaling modulation to alterations of the epigenome. Previously, we reported that two binge-like exposures to EtOH (3 g/kg, ip, 9 h apart) in adolescent rats abolished long-term synaptic depression (LTD) in hippocampus slices, induced learning deficits, and increased N-methyl-D-aspartate (NMDA) receptor signaling through its GluN2B subunit after 48 hours. Here, we tested the hypothesis of EtOH-induced epigenetic alterations leading to modulation of GluN2B and GluN2A NMDA receptor subunits. Forty-two days old rats were treated with EtOH or the histone deacetylase inhibitor (HDACi) sodium butyrate (NaB, 600 mg/kg, ip) injected alone or 30 minutes before EtOH. After 48 hours, learning was tested with novel object recognition while synaptic plasticity and the role of GluN2A and GluN2B subunits in NMDA-fEPSP were measured in CA1 field of hippocampus slices. LTD and memory were impaired 48 hours after EtOH and NMDA-fEPSP analysis unraveled changes in the GluN2A/GluN2B balance. These results were associated with an increase in histone deacetylase (HDAC) activity and HDAC2 mRNA and protein while Ac-H4K12 labelling was decreased. EtOH increases expression of HDAC2 and mRNA level for GluN2B subunit (but not GluN2A), while HDAC2 modulates the promoter of the gene encoding GluN2B. Interestingly, NaB pretreatment prevented all the cellular and memory-impairing effects of EtOH. In conclusion, the memory-impairing effects of two binge-like EtOH exposure involve NMDA receptor-dependent LTD deficits due to a GluN2A/GluN2B imbalance resulting from changes in GluN2B expression induced by HDAC2.

KEYWORDS
epigenetic, ethanol, long-term depression
INTRODUCTION

For more than a decade, the effects of drugs of abuse in the central nervous system have been shown to involve epigenetic changes on either histones or DNA. Concerning ethanol (EtOH), for example, a study showed in 2004 that chronic EtOH exposure increases expression of the GluN2B NMDA receptor subunit in cortical neurons through DNA demethylation. Such epigenetic changes induced by EtOH have also been described during neurodevelopment, adolescence, and in the development of EtOH addiction. Specifically, epigenetic changes have been reported in fetal alcohol spectrum disorder linking the genome, the environmental conditions, and the neurodevelopmental outcomes but also ensuring the transmission of EtOH-related pathologies across generations. ETOH-induced epigenetic modifications have also been implicated in the acute effects of EtOH in cerebral cortex or in chronic EtOH exposure during adult life or following intermittent subchronic EtOH exposure during adolescence. For instance, adolescent intermittent EtOH exposure in rats increased whole hippocampus histone deacetylase (HDAC) activity associated with a decrease in histone H3 acetylation level at adult age, leading possibly to decrease in brain-derived neurotrophic factor (BDNF) expression. In rat hippocampal-entorhinal cortex brain slice cultures, EtOH exposure for 96 hours decreases HDAC1/4 activity.

At behavioral level, our laboratory showed that EtOH-induced sensitization in mice was accompanied with a reduction in striatal HDAC activity and an increase in H4 acetylation level in the core of nucleus accumbens. Interestingly, the histone deacetylase inhibitor (HDACi) sodium butyrate (NaB) targeting HDAC I and II family classes prevented or reversed EtOH-induced behavioral sensitization potentially through specific gene regulations in both the striatum and the prefrontal cortex. Furthermore, NaB treatment reduced motivation to drink EtOH and EtOH consumption in the postdependent-state model of EtOH addiction in rats. Animal treatment with MS-275, an HDACi targeting class I HDAC, reduced motivation to consume EtOH and relapse in heavy drinking rats and reduced binge-like EtOH drinking in mice, revealing that modulation of epigenetic processes with HDACi is capable to decrease EtOH addiction-related phenotype. In this context, it has been proposed that targeting epigenetic processes that modulate synaptic plasticity may indeed yield to novel treatments for EtOH addiction. Interestingly, among the 18 isoforms of HDAC, the HDAC2 isoform seems to be particularly implicated in the effects of EtOH as EtOH-prefering rats knockdown for HDAC2 in the amygdala showed less anxiety-like behavior and less voluntary EtOH drinking.

Epigenetic modulations are not implicated only in pathologies such as EtOH addiction, but they also participate to physiological regulations, in particular during learning and memory processes. Memory consolidation is known to require protein synthesis. Indeed, gene expression is controlled by cofactors associated with transcription factors and repressors through interaction with transcription machinery, chromatin remodeling, and/or chemically modified histones. Hence, histone acetylation by histone acetyltransferase (HAT) has been suggested a key mechanism not only in memory consolidation but also in memory reconsolidation, being involved in retrieval-dependent memory updating through the regulation of specific molecular and cellular processes. Neonatal exposure to isoflurane, for example, induces long-term memory impairment in mice because of dysregulated histone acetylation in the hippocampus that can be attenuated by the HDACi trichostatin A (TSA). Inhibiting HDAC increased long-term potentiation (LTP), one of the neuronal synaptic plasticity signals linked to learning and memory processes at cellular level in the hippocampus, while learning performance in novel object recognition (NOR) test, an hippocampus-dependent task is ameliorated. Other types of learning are also increases after inhibition of HDAC such as inhibitory avoidance in relation with the amygdala.

Binge drinking, a pattern of drinking leading to intoxication in a single short session, is a serious but preventable public health problem. It is the dominant type of alcohol misuse in adolescents and is associated with memory and cognitive impairments in both humans and animals. However, whether epigenetic mechanisms are involved in the memory-impairing effects of only few binge drinking episodes remains unclear. In this context, we recently reported that only two exposures to EtOH (3 g/kg, ip, 9 h apart, 2 g/L blood EtOH content) specifically reduced long-term synaptic depression (LTD), the other synaptic plasticity signal related to learning and memory in hippocampus slices of adolescent rats after 48 hours. However, the mechanism(s) by which LTD is impaired after binge drinking–like exposure is not established although a first attempt was made since we demonstrated a concomitant increase in the involvement of the NMDA receptor subunit GluN2B to the NMDA field excitatory postsynaptic potentials (EPSPs) in the hippocampus. According to the literature, epigenetic appears as a good candidate in explaining the 48-hour delayed effects of EtOH on synaptic plasticity. Therefore, in the present study, we tested the hypothesis that epigenetic mechanisms may play a role in the plasticity and memory-impairing effects of two EtOH binge drinking–like episodes. We evaluated the HDACi, NaB, to prevent the effects of the two EtOH exposures on synaptic plasticity from acute hippocampus slices, on learning performances with NOR, and on HDAC activity, HDAC2 protein, and mRNA levels as well as histone acetylation (Ac-H3K9 and Ac-H4K14) with immunohistochemistry, qPCR, and western blotting methods. To further define the mechanisms by which EtOH and epigenetics may alter LTD, we analyzed the sensitivity of NMDA-EPSPs to GluN2A and to GluN2B subunit antagonists and performed flow cytometry and ChIP-qPCR analysis of GluN2A and GluN2B gene promoter to demonstrate HDAC2 regulation in isolated CA1.

MATERIAL AND METHODS

Experiments were performed following the European Community guiding principles for the care and use of animals (2010/63/UE, CE Off. J. 20 October 2010), the French decree no. 2013-118 (French Republic Off. J., 2013), and the local institution ethics committee rules (CREMEAP, University of Picardie Jules Verne).
2.1 Drugs

EtOH (VWR, Strasbourg, France) was diluted in saline (0.9% NaCl) at 15% (v/v) final concentration and intraperitoneally (ip) injected. NaB (Sigma Aldrich, Saint Quentin Fallavier, France) was dissolved in saline and administered ip at 600 mg/kg, alone at 8:00 AM and 5:00 PM on day 1, or 30 minutes before each EtOH injections. TSA from Hellobio (UK) was prepared in phosphate-buffered saline (PBS) (20% dimethylsulfoxide [DMSO]) as stock solution, diluted at final dose in saline (2 mk/kg), and then ip injected. The timeline of treatments and experiments is illustrated in Figure 1.

2.2 Ethanol and NaB exposure

All experiments were performed on male Sprague Dawley rats during late adolescent period (42-55 d). The timeline of experiments is illustrated in Figure 1. Animals received one of the following treatment: (i) NaCl group: two ip injections of saline at 8:00 AM and 5:00 PM on day 1 (ie, administered 9 h apart) with each injection being preceded by 30 minutes with one ip injection of saline; (ii) EtOH group: two ip injections of EtOH (3 g/kg; 15% v/v) following the same timeline as for NaCl with each injection being preceded by 30 minutes with one ip injection of saline; (iii) NaB group: two ip injections of saline following the same timeline as for NaCl with each injection being preceded by 30 minutes with one ip injection of NaB (600 mg/kg); and (iv) EtOH + NaB group: two ip injections of EtOH (3 g/kg) administered 9 hours apart as for NaCl with each injection being preceded by 30 minutes with one ip injection of NaB (600 mg/kg). All measurements were performed after 48-hour delay.

2.3 Slices and electrophysiology

Slices and electrophysiology data were obtained as previously described. Briefly, after halothane anesthesia and decapitation, the brain was immersed in artificial cerebrospinal fluid (aCSF, 4°C) and serially cut with a vibratome (Leica VT1000E, Rueil-Malmaison, France) to obtain 400-μm-thick slices from dorsal hippocampi, which were stored (≥60 min) in carbogen (95% O2/5% CO2; pH 7.2-7.4; 28°C) gassed aCSF of the following composition (mM): NaCl 125, KCl 3, NaH2PO4 1.25, CaCl2 2.3, MgCl2 1.3, NaHCO3 25, and glucose 10. Recorded slices were transferred to a recording chamber perfused at a rate of 6 mL/min. A bipolar stimulating electrode (Phymep, Paris, France) was placed on the Schaffer collateral and used at 0.033 Hz frequency (square pulse, 100 μs duration) for LTD experiments and 0.1 Hz for LTP. Field potential responses (either population spike or fEPSPs) were collected with 3M NaCl (1-3 MΩ) filled glass microelectrodes into the pyramidal cell body layer of CA1 or in the stratum radiatum. For each slice, an input/output relationship was performed to determine the maximal response from which test stimulus strength was set to 50% to 60% of this maximum. Signals were amplified (grass amplifier, ×1000-2000), filtered (1-3 KHz), and recorded on computer with Signal software (CED, Cambridge, UK) for off-line analysis. To induce LTD, we used a train of pair of pulses separated by 200 milliseconds and delivered 900 times at 1 Hz of pLFS200-900. LTP was induced with 2 × 1-second duration stimulation at 20 Hz, separated by 10 seconds. Plasticity was recorded for 60 minutes. NMDA-fEPSPs were pharmacologically isolated (zero-Mg2+ aCSF + 50μM bicuculline + 10μM CNQX, to block GABA-A and AMPA receptors, respectively), and 5μM Ro 25-6981 antagonist was used to test the role of GluN2B subunit and 20nM NVP-AAM077 antagonist for GluN2A subunit.34 At the end of recording, 20μM AP-5 was added. Results are expressed as percent change relative to baseline value of the population spike amplitude or the EPSPs slope. Drugs were applied sequentially and alternatively from one recording to another. Only slices demonstrating 10 to 15 minutes of stable baseline recording were used, and measurements were averaged every minute. For electrophysiology, “n” is the number of slices tested and “N” is the number of rats used. Average raw traces either for population spike or for EPSPs were made from 30 to 60 sweeps.

2.4 Western blot experiments

Ten rats were randomly assigned to control (NaCl, n = 5) or EtOH (n = 5) group. One rat was discarded for technical problems, and

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FIGURE 1 Timeline of the experiments
experiment was duplicated for reproducibility. Cytoplasmic proteins of isolated CA1 of hippocampus were extracted with Nonidet P40 (NP40) buffer (1% NP40, 50mM Tris pH 7.5, 10% glycerol, 150mM NaCl, 1mM EDTA, 100mM Na₂VO₄, 0.5mM phenylmethanesulphonyl fluoride [PMSE], 5 mg/mL aprotinin, 5 mg/mL leupeptin, and 2 mg/mL pepstatin) containing protease and phosphatase inhibitors. Nuclear proteins were extracted with NP40 containing protease and phosphatase inhibitors and 1% TritonX100 (Sigma Aldrich). Equal amounts of each protein sample were separated by electrophoresis on SDS-PAGE, blotted onto nitro-cellulose membranes (Bio-Rad, Munich, Germany), and blotted with antibodies against HDAC2 (ab32117, Abcam) and GAPDH (#2118, Cell Signaling Technology, Cambridge, UK and Montigny-le-Bretonneux, France). Blots were developed with the Clarity™ chemiluminescence (ECL) system (Bio-Rad).

2.5 | HDAC activity assay

Ten rats were randomly assigned to control (NaCl, n = 5) or EtOH (n = 5) group. HDAC activity was measured in whole hippocampus using the luminase HDAC-Glo™ I/II Assay and Screening System (Promega, Charbonnières-les-bains, France). One μg of nuclear proteins of hippocampus extracts were diluted in 100 μL of the HDAC-Glo™ I/II Buffer in a 96-well white-walled LUMITRAC™ plate. After adding HDAC-Glo™ I/II Reagent, luminescence was measured on a microplate reader (Tecan, Switzerland) every 90 seconds, and quantification was performed after 20 minutes, i.e., at maximum luminescence intensity. Experiment was performed in triplicates.

2.6 | RNA extraction and quantitative real-time PCR

Total RNA of isolated CA1 was extracted using Maxwell®RSC Simply RNA Tissue (Promega), and 5 μg of total RNA was reverse transcribed into cDNA with multiscribe reverse transcriptase (Applied Biosystems, Villebon-sur-Yvette, France). Quantitative PCR was performed according to the GoTaq® RT-qPCR Systems protocol (Promega) using the following primers: HDAC2 For 5′-GGCTCAACGGATTCTGCTACG-3′ and GAPDH For 5′-GTTCACGGCACAGTCAAGG-3′.

2.7 | Immunohistochemical labelling of acetylated H3 and H4 (Ac-H3, Ac-H4) and HDAC2

Forty-two days old rats (n = 40) were randomly assigned to the NaCl, EtOH, NaB, and EtOH + NaB groups and anesthetized with pento- barbital (60 mg/kg, ip) plus heparine (2500 UI, ip). Vascular bed was flushed using the aorta with 75 mL of heparinized 0.9% saline and 200 mL of 4% paraformaldehyde (PFA) in 0.1M PBS (T° 4°C, pH 7.4). Brains were removed, postfixed in 4% PFA overnight, and placed into a 30% sucrose cryoprotection solution for a week. Fifty-micrometer frozen coronal sections of dorsal hippocampus (Bregma –2.80 mm to Bregma –3.60 mm) were obtained with a cryo- stat and put in cryoprotective solution (ethylene glycol 30%; Sucrose 30%, NaCl 0.9%, in PBS 1×) and stored at –20°C. Floating sections were immunostained for Ac-H3K9, Ac-H4K12, and HDAC2 as previously described. Slides were rinsed in Phosphate buffered saline with Tween-20 (PBST) (PBS 1× [ref 8461.0005, ChemSolute, Bruhath, France], 0.2% Triton, pH 7.4) and incubated (15 min) with 3% hydrogen peroxide in methanol to quench endogenous peroxidases and then rinsed in PBST. Incubation for 24 hours at 4°C in a blocking solution (PBS 1×, bovine serum albumin [BSA] 0.1%, TritonX100 0.2%, Goat serum 2% [BioWest, Nuaillé, France]) followed with, respectively, a rabbit anti-acetyl-histone H3 (Lys9) antibody (Upstate, Lake Placid, New York) diluted (1/1000); a rabbit monoclonal anti-acetyl-histone H4 (Lys12) antibody (Millipore, Guyencourt, France) diluted (1/7500); a rabbit monoclonal anti-HDAC2 antibody diluted (1/500) (ab32117, Abcam).

For HDAC2 labelling, heat-mediated antigen unmasking was performed in sodium citrate buffer (sodium citrate 10mM, Tween20, 0.05%, pH 6) for 3 × 30 seconds in microwave before first antibody incubation. Slides were rinsed in PBST and processed for Ac-H3, HDAC2 (Vectastain ABC Elite kit; SK-4100, Vector Laboratories, Burlingame, California), and Ac-H4 labellings with biotin-conjugated goat anti-rabbit secondary antibody 1/2000 (111-065-144 Jackson ImmunoResearch, Cambridge, UK) Immunolabelling was computerized through Mercator® (Explora Nova, France) image analysis system. All images were acquired using the same light level by using a 20× objective lens and analyzed by a “blind” experimenter. A basal threshold was established and applied to all images. Subsequently, the grayscale digital image was classified into positive staining areas and background. Cells with immunoreactivity above this threshold were counted as immuno-positive cells. Immuno-positive cells were counted and normalized in areas of 100 000 μm².

2.8 | Flow cytometry analysis

Methods for flow cytometry were adapted from Benoit et al.36 Isolated CA1 brain tissue was dissociated into single-cell suspensions. The extracellular matrix was enzymatically digested using the Adult Brain Dissociation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), while gentleMACS™ Dissociator with heaters was used for mechanical dissociation during the on-instrument enzyme incubation. Single-cell suspension was labelled with FITC-conjugated Ab specific for NeuN (ab190195, Abcam), PE-conjugated Ab for HDAC2 (sc-9959, Santa Cruz), acH4K12 (ab46983, Abcam), biotinylated GluN2A Ab (24405A, Novus), Alexa fluor 647-conjugated Ab for GluN2B (47611AF647, Novus) to flow cytometry analysis. For fluorescence-activated cell sorting (FACS) analysis, cells were incubated with antibodies in staining buffer (PBS and 2.5% FCS) for 30 minutes and washed. Intracellular HDAC2 and acH4K12 staining were also performed. Cells were subsequently fixed with 1% PFA for 15 minutes at 4°C, permeabilized with 0.1% Triton, 1% BSA in PBS for 30 minutes at 4°C, and then incubated for 30 minutes at 4°C with PE-conjugated anti-HDAC2 or anti-acH4K12 in permeabilization buffer. Labelled cells were analyzed on a MACSQuant cytometer running MACSQuantify software (Miltenyi Biotec) and FlowJo software (Tree Star, Ashland, Oregon).
2.9 | ChIP-qPCR experiments

The chromatin immunoprecipitation (ChIP) assay was performed using high-sensitivity ChIP kit (Abcam) according to the manufacturer’s protocol. The antibodies used were as follows: anti-HDAC2 (ab12169) and anti-AcH4K12 (ab46983) from Abcam. After immunoprecipitation, purified ChIP DNA samples were used for RT-qPCR using the following primers: Nr2a Forward 5'-TCACTGATAGCGCTGCT-3', Reverse 5'-TGGAGGACGTGGATGTCCG-3'; Nr2b Forward 5'-TGGATCCTCGTGAACAGAGA-3', Reverse 5'-CACATC CGAGGCCACACATA-3'. Fold enrichment was calculated to determine interaction of protein to the promoter region using a ratio of amplification efficiency of the ChIP sample over that of nonimmune immunoglobulin (IgG).

2.10 | Novel object recognition

NOR was previously described.²⁹ Forty-two days old animals (n = 40) were allowed to acclimatize to the animal care facility, handled twice a day, and randomly assigned to one of the following groups: NaCl, EtOH, NaB, and EtOH + NaB (n = 10 animals per group). Learning phase took place 48 hours after the first injection. Rats were transferred to the test room 30 minutes before the experiment. The apparatus was a square box with an open top, made of opaque Plexiglas (45 × 45 × 45 cm) and under a 30-lux illumination. The objects were different enough in shape to be distinguished and were too heavy to be displaced by rats and located at the same distance from the wall. After a 10-minute habituation, animals were submitted to a 10-minute learning phase with two objects. Test phase was performed 48 hours later by replacing one object by a new one. Digital video acquisition system (Pinnacle Studio HD v.15 software) was used. We measured the total exploring time spent on the objects during the first 5 minutes of recording (sniffing, licking, or touching the objects with forelimbs). Exploration time was normalized and expressed as a percentage of time spent by rat on novel object compared with the familiar object.

2.11 | Statistics and data presentation

All data are expressed as mean ± SEM. Statistical analyses were conducted using SigmaStat® software (LogiLabo, Paris, France). A two-way repeated measure ANOVA was used on raw data for synaptic plasticity and immunohistolabelling experiments. A two-way ANOVA without repetition was used for EPSPs and NOR experiments. Western blot, qPCR, and enzymatic assay were tested with unpaired Student t test. Post hoc multiple comparisons were conducted using Tukey test. The data normality was also tested and passed. Statistical significance was set at P < 0.05.

3 | RESULTS

3.1 | Effects of two EtOH exposures on synaptic plasticity and reversal with HDACi

We first measured synaptic plasticity in CA1 area of acute hippocampus slice 48 hours after treatment as previously described.²⁹ Concerning LTD, the two-way ANOVA revealed an effect of the EtOH factor (F1,26 = 16.11, P < 0.001), no effect of the NaB factor (F1,26 = 3.0, P = 0.096), and no significant interaction between factors (F1,24 = 1.44, P = 0.242). Post hoc analyses showed a significant decrease of LTD after EtOH. LTD magnitude in control group was −40.9 ± 5.5% of baseline values whereas LTD was −18.1 ± 3.6% (P = 0.001 between groups) after EtOH. Thus, as reported in our previous study, LTD was strongly reduced after 48 hours (Figure 2A). NaB alone had no effects on LTD since values were not significantly different from the control group (NaCl, −40.9 ± 5.5% vs NaB, −43.2 ± 5.8%, P = 0.72; Figure 2B). However, NaB injected as a pretreatment before EtOH prevented significantly the EtOH-induced LTD reduction (Figure 2C). Indeed, LTD magnitude after NaB + EtOH and after EtOH alone reached respectively −31.0 ± 2.3% vs −18.1 ± 3.6% (P < 0.042). In addition, LTD magnitude after EtOH + NaB was not significantly different from NaCl level (−31.0 ± 2.3% vs −40.9 ± 5.5%, respectively, P = 0.06; Figure 2G). In summary, NaB significantly prevented the effects of EtOH on LTD magnitude without being effective when given alone. Similar results were obtained when TSA was used instead of NaB (see Figure S1).

Concerning LTP, two-way ANOVA revealed an effect of the EtOH factor (F1,20 = 10.117, P = 0.005), no effect of the NaB factor (F1,20 = 3.979, P = 0.061), and no significant interaction between factors (F1,18 = 0.164, P = 0.69). Post hoc analyses showed that EtOH alone increased significantly LTP magnitude (Figure 2D and 2H) as already described.²⁹ Thus, LTP magnitude reached 10.6 ± 4.2% for the NaCl group vs 26.0 ± 4.8% for the EtOH group (P = 0.013; Figure 2D and 2H). NaB alone had no effects on LTP as compared with control group (NaB, 3.7 ± 1.6% vs NaCl, 10.6 ± 4.2%, P = 0.3; Figure 2E and 2H). When animals were treated with NaB + EtOH, LTP was not significantly altered as compared with EtOH alone (EtOH + NaB, 15.7 ± 4.0% vs EtOH, 26.0 ± 4.8%, P = 0.09; Figure 2F and 2H). In summary, the increased in LTP magnitude observed after EtOH was not prevented by NaB, and NaB alone was not effective.

We previously reported that reduction of LTD following two EtOH binges was not present if recordings were performed after 8 days delay.²⁹ Here, we tested how long NaB was protective against LTD reduction by EtOH. Recordings after 72 hours showed that LTD was still significantly reduced by EtOH (from −42.3 ± 6.2% in the NaCl group vs −20.8 ± 4.7 after EtOH, P = 0.02) and that NaB still prevented the EtOH effects (from −20.8 ± 4.7 after EtOH to −44.8 ± 6.0 after NaB + EtOH, P = 0.008; Figure 3A1-A2). Thus, pretreatment with NaB fully restored LTD as compared with the NaCl group (EtOH + NaB, −44.8 ± 6.0 vs NaCl, −42.3 ± 6.2, P = 0.8). When recordings were performed 96 hours after the binges, there was no effect of EtOH (NaCl,
−42.3 ± 6.2%, EtOH, −39.0 ± 7.4%, P = 0.7) and thus NaB pretreatment had no effect compared with the NaCl group (EtOH + NaB, −51.4 ± 3.2% vs NaCl, −42.3 ± 6.2%, P = 0.3; Figure 3B1-B2).

3.2 | Sensitivity of NMDA-fEPSPs to GluN2A and GluN2B antagonists

Previously, we reported that NMDA-fEPSP become more sensitive to GluN2B antagonist 48 hours after EtOH. Here, we checked whether NaB prevented this effect, and we extended our work to the analysis of the NMDA-fEPSP sensitivity to an antagonist of GluN2A subunit. Regarding the effects of NVP-AAM077, a GluN2A antagonist, two-way ANOVA revealed an effect of the EtOH factor (F_{1,39} = 7.9, P = 0.008), no effect of the NaB factor (F_{1,39} = 1.7, P = 0.205), and no interaction (F_{1,37} = 1.8, P = 0.486). Post hoc test showed that EtOH reduced significantly NVP-AAM077 effects on NMDA-fEPSPs by 61.3 ± 25.1% (from −22.7 ± 3.2% in control slices to −8.8 ± 2.3% after EtOH, P = 0.007; Figure 4A). After NaB alone, the percent of
inhibition by NVP-AAM077 was similar to that measured in control slices (NaCl, −22.5 ± 3.2 vs NaCl, −22.7 ± 3.2%, P = 0.97; Figure 3A). Finally, pretreatment with NaB before EtOH showed a significant prevention of EtOH effects (EtOH, −8.8 ± 2.3% vs EtOH + NaB, −17.6 ± 2.8%, P = 0.05).

When the GluN2B antagonist Ro25 6981 was tested on NMDA-fEPSPs, two-way ANOVA revealed an effect of the factor EtOH (F_{1,39} = 14.9, P < 0.001), no effect of the NaB factor (F_{1.39} = 3.2, P = 0.08), and an interaction (F_{1.37} = 9.9, P = 0.003). In contrast to NVP-AAM077, EtOH increased the sensitivity to Ro25 6981 by 259.1 ± 57.1% (from −9.6 ± 3.7% in control slices to −34.4 ± 3.5% after EtOH, P < 0.001; Figure 4B). NaB alone had no effect on the sensitivity to GluN2B antagonist (NaB, −14.4 ± 3.9% vs control, −9.6 ± 3.7%, P = 0.378; Figure 3B) and pretreatment with EtOH decreased the effects of EtOH (NaB + EtOH, −17 ± 2.9% vs EtOH, −34.4 ± 3.5%, P < 0.001; Figure 3B). In summary, NMDA-fEPSP become less sensitive to GluN2A antagonist and more sensitive to GluN2B antagonist after EtOH and NaB pretreatment prevents significantly these effects of EtOH while NaB alone had no effects.

Furthermore, the input/output curve established for NMDA-fEPSP was shifted leftward 48 hours after EtOH whereas after NaB + EtOH, the curve resumed to control (Figure 4C). In addition, the input/output curve for NaB alone was similar to that of NaCl (Figure 4C). Finally, the Bernstein-Cooper-Munroe (BCM) theoretical curve in our experimental conditions showed that after EtOH, the threshold to induce LTD was shifted to the left as compared with NaCl whereas after NaB + EtOH, it was shifted back to the right, towards the control curve (Figure 4D).

### 3.3 Effects of HDACi on the memory-impairing effect of EtOH

We next tested the effects of NaB on learning task that we showed previously to be impaired after the two binge-like exposures.29 Thus, NOR was performed in the four groups of animals following the time line depicted in Figure 1. During training phase and in the presence of two objects A and B, all animals spent 50% of their exploratory time on each object (Figure 5A), revealing no spontaneous preference. Object A was explored for 47.9 ± 1.3% of the total exploratory time, and object B was explored for 52.1 ± 1.3% of the total time (P > 0.05 between groups). During test phase (Figure 5B), two-way ANOVA revealed an effect of the NaB factor (F_{1,37} = 8.952, P = 0.005), an effect of the EtOH factor (F_{1,37} = 14.822, P < 0.001), and a significant interaction between factors (F_{1,35} = 15.209, P < 0.001). Post hoc analysis showed that in the group treated with EtOH (Figure 5C), animals spent significantly less time exploring the novel object C than NaCl treated animals (EtOH, 38.0 ± 3.14% vs NaCl, 61.3 ± 3.2%, P < 0.001). Importantly, rats treated with EtOH + NaB spent significantly more time on the novel object than animals treated with EtOH alone (EtOH + NaB, 58.75 ± 3.2% vs EtOH, 38.0 ± 3.14%, P < 0.001), but similar time than the NaCl group (EtOH + NaB, 58.75 ± 3.2% vs NaCl, 61.3 ± 3.2%, P = 0.8). Finally, NaB alone had no effect on learning performance since there was no difference between the NaCl group and the NaB group (NaCl, 61.3 ± 3.2% vs NaCl + NaB, 58.6 ± 2.45%, P = 0.243; Figure 5C).

### 3.4 Epigenetic changes and the effects of HDACi

Both electrophysiology and behavioral data suggest that two EtOH intoxications trigger epigenetic modifications after 48 hours, at a time
when LTD and cognition deficits were detected. Thus, in a first set of experiments, we measured enzymatic activity for the HDAC I/II classes and HDAC2 protein and mRNA levels in NaCl versus EtOH groups (Figure 6) 48 hours after EtOH. The results showed that enzymatic HDAC I/II classes activity in whole hippocampus homogenates was enhanced after EtOH (Figure 6A; NaCl, 28894.6 ± 4909.1 vs EtOH, 36290.3 ± 3997.9, P < 0.05). HDAC2 mRNAs (NaCl, 4.5 ± 0.76 vs EtOH, 5.6 ± 0.96, P < 0.05; Figure 6B) and HDAC2 protein levels (isoform from the HDAC I family) were also increased after EtOH in isolated CA1 area (NaCl, 0.1 ± 0.22 vs EtOH, 1.4 ± 1.1, P = 0.05; Figure 6C). Furthermore, using flow cytometry technic, we found that 48 hours after ETOH, more neurons (NeuN-positive cells) were

FIGURE 4  NMDA-fEPSPs sensitivity to GluN2A and GluN2B antagonists, input/output curve, and BCM-like curve. A. Effects of NVP-AAM077, an antagonist of GluN2A subunit on averaged raw traces of NMDA-fEPSPs in different condition as shown on the left: control (NaCl, n = 9 and N = 4), NaB alone (NaB, n = 8, N = 4), EtOH alone (EtOH, n = 8, N = 5), and EtOH + NaB (n = 11 and N = 6). Traces illustrated the baseline condition (left row, CTRL), the drug effect (middle row), and the superimposed traces (right row). The bar graph illustrates the statistic for the whole populations tested. B. Similar presentation than in (A) but for RO25-6981, a GluN2B subunit antagonist; (NaCl, n = 9 and N = 4), NaB alone (NaB, n = 8 and N = 4), EtOH alone (EtOH, n = 8 and N = 5), and EtOH + NaB (n = 14 and N = 6); vertical calibration: 0.02 mV; horizontal calibration: 20 ms. C, NMDA-fEPSP slope as a function of stimulus intensity showing that after EtOH (black dots), the relationship is shifted to the left compared with control (open dots, NaCl) whereas after EtOH + NaB, the curve resumes to NaCl condition and that NaB had no effect on this relationship (grey dots). D, Bernstein-Cooper-Munro–like curve (BCM) illustrating the capacity to evoke either LTP or LTD as a function of stimulation. Compared with control (NaCl), ETOH shifted the curve leftward, revealing difficulties to evoke LTD. In contrast, after ETOH + NaB, the relationship resumed to control level. Same statistic labelling as in Figure 2 and **P < 0.01
expressing HDAC2 (Figure 6D). This increase was found in both the percent of cells expressing HDAC2 (NaCl, 59.47 ± 4.22% vs EtOH, 74.10 ± 11.0%, \( P < 0.05 \)) and HDAC2 mean fluorescence intensity (NaCl, 3.15 ± 0.51 vs EtOH, 6.17 ± 0.97, \( P < 0.05 \); Figure 6E). To go further, we measured the number of HDAC2 immunolabelled cells as well as acetylated H3 (Ac-H3K9) and H4 (Ac-H4K12) staining in CA1 area (Figure 7). Concerning Ac-H4K12 (Figure 7A), the two-way ANOVA revealed an effect of the NaB factor (\( F_{1,30} = 5.599, P = 0.025 \)), no effect of the EtOH factor (\( F_{1,30} = 1.888, P = 0.18 \)), and no interaction between both factors (\( F_{1,28} = 2.802, P = 0.105 \)). Post hoc analyses showed that EtOH exposure induced a significant decrease of Ac-H4K12 immunolabelling in CA1 (NaCl, 1279.2 ± 34.8 vs EtOH, 1111.1 ± 52.4, \( P = 0.034 \)) and that NaB alone was devoid of effects on Ac-H4 immunolabelling (NaCl, 1279.2 ± 34.8 vs NaB, 1317.4 ± 63.0, \( P = 0.617 \); Figure 7A). However, NaB injected as a pretreatment before EtOH prevented the EtOH-induced Ac-H4K12 expression decrease (EtOH, 1111.1 ± 52.4 vs EtOH + NaB, 1333.9 ± 71.2, \( P = 0.01 \); Figure 7A). Regarding Ac-H3K9, its labelling was unaltered (see Figure S2). Concerning HDAC2 (Figure 7B), two-way ANOVA revealed an effect of the NaB factor (\( F_{1,27} = 13.696, P = 0.001 \)), no effect of the EtOH factor (\( F_{1,27} = 1.49, P = 0.234 \)), and an interaction between factors (\( F_{1,25} = 10.362, P = 0.004 \)). Post hoc analyses showed that EtOH exposure induced a significant increase of HDAC2 immunolabelling in CA1 (NaCl, 998.1 ± 52.2 vs EtOH, 1228.9 ± 58.2, \( P < 0.01 \)) while NaB alone had no effect (\( P = 0.747 \)). Again, NaB injected as pretreatment prevented the EtOH-induced increase in HDAC2 expression (EtOH, 1228.9 ± 58.2 vs EtOH + NaB, 869.2 ± 57.0, \( P < 0.001 \)).

3.5 | EtOH effects on GluN2A and GluN2B expression levels: role of HDAC2 and prevention by NaB

In order to demonstrate that HDAC2 is involved in the effects EtOH on the expression levels of GluN2A and GluN2B subunits of the NMDA receptor, we combined flow cytometry and ChIP-qPCR techniques to isolated CA1. First, RT-qPCR revealed that only GluN2B mRNA levels were increased after EtOH treatment (NaCl, 6.95 ± 0.85 vs EtOH, 8.18 ± 1.10, \( P > 0.05 \) for GluN2A and NaCl, 0.98 ± 0.04 vs EtOH, 1.14 ± 0.60, \( P < 0.05 \) for GluN2B; Figure 8A). The number of neurons expressing GluN2B subunit was increased after EtOH and not that of GluN2A subunit (Figure 8B). More specifically, in GluN2A highly expressing neurons, there was no modulation by either EtOH or NaB applied alone or in combination since both the percent of cells expressing GluN2A and mean fluorescence intensity were not altered (Figure 8C left). In contrast, the percent of cells expressing GluN2B was increased after EtOH (NaCl, 45.48 ± 4.88% vs EtOH, 64.68 ± 6.22, \( P < 0.05 \)) and in GluN2B mean fluorescence intensity (NaCl, 1.00 ± 0.11 vs EtOH, 1.99 ± 0.40, \( P < 0.05 \); Figure 8C right). Importantly, NaB pretreatment decreased EtOH effects on the percent of cells expressing GluN2B and on GluN2B mean fluorescence intensity although not significantly (Figure 8C right) while NaB was not effective when tested alone (NaCl, 45.48 ± 4.88% vs NaB, 49.53 ± 7.23%, \( P > 0.05 \) and NaCl, 1.00 ± 0.11 vs NaB, 1.18 ± 0.20, \( P > 0.05 \)). Next, we analyzed GluN2A and GluN2B genes promoter enrichment using ChIP-qPCR. Results (Figure 9) showed that there was no enrichment by HDAC2 of GluN2A gene promoter but that...
GluN2B gene promoter was enriched with HDAC2. Again, this latter effect was prevented by NaB pretreatment.

4 Discussion

This study confirms and extends our previous work demonstrating that 48 hours after two binge-like EtOH episodes, LTD—a major form of synaptic plasticity related to learning and memory— is significantly reduced in the hippocampus of adolescent rats, leading to learning deficit. Here, we demonstrated that two binge-like exposures modulate the involvement of both GluN2A and GluN2B subunits of the NMDA receptors in synaptic transmission resulting in an increase in network intrinsic excitability and changes in synaptic plasticity. We further demonstrated EtOH-induced upregulation of GluN2B subunit, HDAC2 expression level and activity in the hippocampus with a parallel decrease in the level of Ac-H4, concomitant to LTD abolition and learning deficit. Furthermore, only the GluN2B gene promoter was reduced in the hippocampus of adolescent rats, leading to learning deficit.
enriched by HDAC2 after EtOH. Consequently, pretreatment with an HDACi prevented all the effects of EtOH on fEPSPs, LTD, behavior, cellular, and epigenetic effects. Therefore, our results demonstrated that two episodes of EtOH intoxication in adolescent rat disturbed synaptic plasticity in the hippocampus leading to cognitive deficits. Finally, our results clearly demonstrated that the memory-impairing effects of two binge-like EtOH exposures involve NMDA receptor-dependent LTD deficits due to a GluN2A/GluN2B imbalance resulting from changes in GluN2B expression induced by HDAC2.

### 4.1 | Alterations in epigenetic processes after two binge-like ethanol exposures

The level of Ac-H4K12 was significantly decreased in CA1 area of the hippocampus whereas the level of Ac-H3K9 was unchanged 48 hours after the two EtOH exposures. Importantly, expression level of HDAC2, the enzyme that deacetylates histone 4, the number of cells positively labelled for HDAC2, HDAC2 mRNA level, and enzymatic activity of HDAC I/II classes were concomitantly increased. Altogether, these data revealed that the K12 site of histone 4 is regulated by EtOH as compared with the K9 site of histone 3 and that the decrease in Ac-H4K12 is probably related to the increase in expression level and enzymatic activity of HDAC2 in CA1. This later assumption is supported by previous results obtained in HDAC2 knockout mice displaying an increase in Ac-H4K12 and Ac-H4K5 levels but not in Ac-H3K14 level. Several previous studies have shown epigenetic changes after EtOH exposure. For example, 6 hours following 3 g/kg ip EtOH injection in C57BL/6J mice, both Ac-H3K9 and Ac-H4K14 were increased in the hippocampus while expression level of HDAC2 was decreased in the cerebral cortex. Similarly, in the caudate putamen of mice, 1 hour after acute ip EtOH (2.5 g/kg) administration, western blotting showed a reduction levels of class I HDACs 1-3 isoforms, with no changes in the prefrontal cortex. Thus, shortly after EtOH treatment, HDAC activity is reduced and acetylated forms of histones are augmented. However, our results were obtained 48 hours after EtOH intoxication, eg, in the absence of plasmatic EtOH. Fifty days after two weeks of chronic intermittent ethanol (CIE) exposure during adolescence in rat, HDAC activity increases in the whole hippocampus accompanied with a significant decrease in Ac-H3K9 immunolabelling in all areas except the DG. In accordance with these data, we found an increase in HDAC I/II classes activity and a higher HDAC2 expression level, accompanied with a decrease in Ac-H4K12 in CA1. Indeed, epigenetic modulation appears to be region dependent, as well as dependent upon HDAC isotypes and histone isoforms. In this context, differences with other studies may come from the pattern of EtOH exposure, ie, two EtOH exposures vs sub-chronic CIE for 2 weeks and/or from the time point of measurements (48 h vs 50 d, for example). A study performed after 7 days withdrawal from a 10-day binge exposure in C57BL/2J mice showed that acute EtOH administration reduced HDAC activity and upregulated Ac-H4K12 levels in the hippocampus.
H4K12 in the nucleus accumbens proportionally to the decrease of striatal HDAC activity. Altogether, these results suggest a sequential change in different epigenetic markers along the duration of EtOH exposure, during withdrawal, and at different time points following EtOH exposure revealing a constant and highly dynamic epigenetic regulation. In addition, ChIP-qPCR analysis allowed us to point GluN2B gene promoter as a specific target of EtOH exposure whereas GluN2A gene promoter is not targeted. In total, our study demonstrated that epigenetic regulation of gene expression occurred at short term after only two EtOH intoxications in the hippocampus and suggest that such modifications of the epigenome may be related to LTD abolition in CA1 area and thus to EtOH-related cognitive deficits. This assumption is supported by our measurements performed with NaB, an HDACi, injected before EtOH, which revealed a significant prevention of EtOH effects on epigenetic modifications. Specifically, the EtOH-induced increase in HDAC2 isoform and the decrease in Ac-H4 labelling were both prevented following NaB pretreatment. Therefore, we may propose that induction of HDAC2 by EtOH participates to the effects of EtOH on synaptic plasticity in CA1 area of the hippocampus.

FIGURE 8  Modulation of GluN2B expression in neuronal cells. A, RT-qPCR for nr2a and nr2b, in NaCl (white bar) and EtOH groups (black bar) (N = 6; *P < 0.05). B, GluN2A and GluN2B expression analyzed with flow cytometry in neuronal cells (NeuN+). GluN2A expression (left) is not changed after either EtOH (red) or EtOH + NaB (dashed redline) treatment compared with control (NaCl, black) whereas GluN2B expression (right) increased in the EtOH group (red) and is slightly reduced in the EtOH + NaB group (dashed redline). C, Percentage of high-expressing GluN2A cells among the neuronal cells (NeuN+) in the different experimental groups (top left) with corresponding mean fluorescence intensity for GluN2A (bottom left). Similar measurements for GluN2B on the right side. N = 6 in each groups and *P < 0.05. Note that only the GluN2B signal (both in % of cells and in mean fluorescence intensity) was positively modulated by EtOH and prevented by NaB pretreatment.
with memory impairment induced by scopolamine or aging, reveal an impairment of memory. High levels of HDAC2 have been associated using an HDACi while Hdac2 knockout mice did not show such and synaptic plasticity impairment related to low memory perfor-
ifically, it was shown that overexpression of HDAC2 induces structural and HDAC is associated with learning and memory processes. Specif-
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4.2 | HDAC2 and memory

A large body of work brought evidences that epigenetic changes play a role in memory formation and maintenance, notably in hippocampus-dependent learning tasks including NOR. Our results showed that the memory-imparing effects of ETOH measured in NOR 48 hours after ETOH were prevented by NaB. Importantly, NaB had an effect only when coapplied with ETOH since NaB alone was not effective. It is known that the level of both acetylated histone and HDAC is associated with learning and memory processes. Specif-
ically, it was shown that overexpression of HDAC2 induces structural and synaptic plasticity impairment related to low memory perform-
ances. Conversely, these authors reversed the observed effects using an HDACi while Hdad2 knockout mice did not show such impairment of memory. High levels of HDAC2 have been associated with memory impairment induced by scopolamine or aging, revealing further the involvement of high HDAC2 level in degraded memory processes. HDAC2 however shows a relative selectivity in terms of memory since loss of HDAC2 accelerates extinction rate of fear responses and conditioned taste aversion and enhanced attention performance without impacting episodic memory or motor learning.

Here, we found an increase in HDAC2 expression level 48 hours after ETOH binge associated with cognitive deficits in NOR and with loss of LTD. Our results are thus in accordance with the literature showing that HDAC2 is an important factor mediating epigenetic blockade of synaptic plasticity involved in learning and memory. Interestingly, the use of an HDACi targeting HDCA2 was shown to attenuate remote memory (ie, month-old), suggesting such pharmacological strategy could be useful to treat long-lasting disturbing memory such as “pathological memory” linked to a history of addiction. Similarly, in our experimental conditions, LTD was preserved after NaB pretreatment, and this was paralleled by the prevention of cognitive performance in NOR. Noteworthy is the fact that NaB protective effect was visible the time ETOH was effective in decreasing LTD magnitude (ie, for 72 h) and disappeared with the reversibility of ETOH effects. Furthermore, the same prevention effects on LTD were found with TSA, another HDACi, revealing that NaB effects are not related to nonspecific or potential side effects of the drug. In summary,
two binges of ETOH during adolescence in rat induced epigenetic changes in the hippocampus associated with synaptic plasticity disturbances and cognitive deficits. However, the link between HDAC2 overexpression and a specific blockade of LTD is lacking at that point. Nonetheless, our measurements of NMDA-fEPSP sensitivity to GluN2A and GluN2B antagonists with and without NaB and the use of both flow cytometry and ChIP-qPCR approaches may help understanding the relation between HDAC2, ETOH, and synaptic plasticity in the hippocampus.

4.3 | Modulation of GluN2A and GluN2B subunits by ETOH and HDACi

Interestingly, we found that epigenetic modifications by two episodes of ETOH intoxication were accompanied by changes in NMDA-fEPSPs sensitivity to antagonists of GluN2A and GluN2B subunits of the NMDA receptor. In control animals, NMDA-fEPSP was more sensitive to GluN2A than to GluN2B antagonist, suggesting albeit indirectly that NMDA synaptic transmission involved more GluN2A than GluN2B containing NMDA receptors. This result is consistent with the literature indicating a major contribution of GluN2A subunit at adult age than GluN2B subunit in glutamatergic synapses. The results after ETOH confirmed and extended our previous work showing an increase of NMDA-fEPSP sensitivity to GluN2A antagonist. This effect was furthermore accompanied with a decrease in the sensitivity to GluN2A antagonist. These two observations suggest that GluN2B is particularly sensitive to ETOH modulation and that ETOH also modulates significantly the sensitivity to a GluN2A antagonist. However, such modulation of GluN2B and GluN2A by ETOH was not found at the mRNA levels or in the number of cells expressing those subunits. Indeed, only mRNA levels for GluN2B increased after ETOH as well as the number of cells expressing it whereas GluN2A mRNA levels, expression, and number of cells expressing this subunit were all unaltered. These findings suggest ETOH-induced alterations in NMDA-fEPSP sensitivity to GluN2A and GluN2B antagonists through the modulation of GluN2B expression levels only. In this context, the decrease of NMDA-fEPSP sensitivity to GluN2A antagonist was
probably indirect, because of the increase in GluN2B expression. Interestingly, the use of NaB prevented EtOH-induced alteration in NMDA-EPSP pharmacological sensitivity but also in EtOH-induced increased in the number of GluN2B positive neurons without altering the results for GluN2A. Furthermore, ChIP measurements clearly revealed a modulation of the sole GluN2B gene promoter, which was reversed in the presence of NaB. In consequence, it could be that EtOH effects on glutamatergic synaptic transmission are expressed via the modulation of the relative contribution of the two NMDA receptor subunits. Thus, measuring the sensitivity to antagonists of these two subunits allowed us to express our results in terms of GluN2A-to-GluN2B ratio in the different experimental conditions. Hence, GluN2A-to-GluN2B ratio decreased after EtOH compared with control, whereas after NaB + EtOH, this ratio increased towards control level because of the preventive effects of NaB. Importantly, changes in GluN2A-to-GluN2B ratio dictate the capacity of a synapse to perform plasticity according to the theoretical learning rule established by Bienenstock et al. Our measurements further support the notion that synaptic plasticity in the hippocampus after EtOH and following EtOH + NaB treatment is controlled by metaplasticity rules (see Figure 4D and also Silvestre de Ferron et al).

However, our results do not take into account that changes measured in our experiments may reflect not only alterations in subunit expression levels but also a reorganization of GluN2B subunit location between synaptic and perisynaptic compartments. Indeed, GluN2B-containing NMDA receptors are highly mobile in the synapse in different conditions. Furthermore, although the level of HDAC2 expression is highly associated with structural plasticity in the hippocampus, an interaction between HDAC2 and synaptic transmission at cellular level cannot be ruled out. Indeed, HDAC2 overexpression suppresses excitatory synaptic function in a cell-autonomous manner in CA1 pyramidal neurons while enhancing GABA-dependent inhibitory transmission, suggesting a role for inhibitory transmission in the LTD reduction 48 hours after EtOH exposure in the present study. Hence, a hypothesis that awaits to be tested is the involvement of GABA inhibition in the blockade of LTD related to the increase in HDAC2 in the hippocampus.

Another result was that NaB tested alone had no effect on NMDA-EPSPs sensitivity to the two antagonists, paralleled with lack of effect on learning capability and LTD magnitude and also on number of cells positive for either GluN2B or GluN2A. Discrepancies with previous studies reporting a positive effect of NaB on learning tasks and synaptic plasticity may come from the time point analyzed, the mode of drug injection, or the age of the animals. Indeed, LTP was shown to be enhanced with TSA when it was acutely tested (ie, bath applied) onto the hippocampal slice obtained, furthermore, from relative young animals (21 days old). Cognitive function that has been shown to be improved, notably in NOR, was tested 24 hours after NaB injection.

In conclusion, binge drinking–like exposure to EtOH during adolescence in rats increases GluN2B subunit through the regulation of its gene promoter by HDAC2. This in turn will induce an imbalance with GluN2A subunit in glutamatergic transmission, leading to a blockade of LTD in the hippocampus and to memory impairment.

ACKNOWLEDGEMENTS

I.D. is supported by INSERM and Région Hauts-de-France. G.F. is supported by the French Ministry of Research, S.P. is holder of a Chair of Excellence “Synaptic plasticity” funded by Région Hauts-de-France. We thank Pr Loïc Garçon for giving us access to the flow cytometry technique.

ORCID

Mickael Naassila https://orcid.org/0000-0002-9788-0918
Catherine Vilpoux https://orcid.org/0000-0003-2462-2878
Olivier Pierrefiche https://orcid.org/0000-0003-1427-9332

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**How to cite this article:** Drissi I, Deschamps C, Fouquet G, et al. Memory and plasticity impairment after binge drinking in adolescent rat hippocampus: GluN2A/GluN2B NMDA receptor subunits imbalance through HDAC2. *Addiction Biology*. 2019;e12760. [https://doi.org/10.1111/adb.12760](https://doi.org/10.1111/adb.12760)