SUPPLEMENTAL INFORMATION

MATERIALS AND METHODS

Materials Trichostatin A (TSA) and 5-azacytidine (5-AzaC) were purchased from Sigma. MS275 (Entinostat) and suberoylanilide hydroxamic acid (SAHA, Vorinostat) were purchased from Chemie Teck. Anti-acetyl H4 (06-866, acetyl-Lys-5, -8, -12, and -16) antibody was obtained from Millipore. Anti-H4 (2592) antibody was obtained from Cell Signaling Technology. Anti-Dnmt1 (ab13537) antibody was obtained from Abcam. Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sc25778) antibody, as well as the horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology. Enhanced chemiluminescence (ECL) reagents were purchased from GE Healthcare. NuPAGE™ Bis-Tris precast gels were purchased from Invitrogen. Bicinchoninic acid (BCA)™ protein assay kit was obtained from Pierce. Complete™ mini, an EDTA-free protease inhibitor cocktail, was purchased from Roche. Phosphatase inhibitor cocktails 2 and 3 were obtained from Sigma-Aldrich.

Animals Male C57Bl/6J mice were obtained from Jackson Laboratories (8-9 weeks). Mice were individually housed under a 12-hour light/dark reverse cycle (lights off at 10:00 AM). Male Long-Evans rats obtained from Harlan (280-300 g at the beginning of the experiment) were individually housed under a regular 12-hour light/dark cycle (lights on at 7:00 AM). Mice/Rats had food and water available *ad libitum*, and were kept in conditions of constant temperature (23°C) and humidity (50%). All animal procedures in this report were approved by the Gallo

Center Institutional Animal Care and Use Committee and were conducted in agreement with the *Guide for Care and Use of Laboratory Animals* (National Research Council, 1996).

Preparation of solutions For the systemic administration in mice, TSA, MS275, SAHA and 5-AzaC were dissolved in 20% (v/v) DMSO (Sigma-Aldrich) and saline solution (0.9% NaCl, w/v; Hospira). For the systemic administration in rats, SAHA was dissolved in 15% DMSO and saline solution. The vehicles used for control treatments were 20% DMSO or 15% DMSO solutions, respectively. All drugs were administered intraperitoneally (i.p.) at a volume of 1 ml per 100 g in mice, or 1 ml per 200 g in rats. Alcohol solution was prepared from ethyl alcohol absolute anhydrous (190 proof) diluted to 20% (v/v) in tap water. Saccharin and sucrose (Sigma-Aldrich) were dissolved in tap water.

Systemic administration of inhibitors Mice/Rats were habituated to the i.p. administration procedure by a daily injection of saline for 3 days before the beginning of experiments. It has been previously studied that 5-AzaC is most effective when administered repeatedly in animal models of depression[1]. We therefore used a similar regime, in which mice received 3 separate i.p. administrations of either the drug or the corresponding vehicle 24, 18, and 2 hours before the beginning of the test session (Figure S2a). HDAC inhibitors have significant effect on the central nervous system and on behavior when they are administered 2 hours before the beginning of the test[2-4]. Thus, to test TSA, SAHA and MS275 exposure effects, mice/rats received one i.p. administration of the drugs or their respective vehicles 2 hours before the beginning of the test session (Figure S2b-d).

Intermittent access to 20% alcohol 2-bottle choice in mice The procedure was adapted from[5]. Mice were acclimatized for 2 weeks to the reverse dark cycle housing conditions and then had concurrent access to one bottle of 20% alcohol and one bottle of water for 24 hours, starting at 12:00 PM (i.e., 2 hours after the lights turned off) on Mondays, Wednesdays and Fridays for 8 weeks before the beginning of the experiments. The placement (left or right) of each solution was alternated between each session to control for side preference. Alcohol intake (g/kg of body weight) and the preference ratio for alcohol solution (volume of alcohol solution intake/total volume of fluid intake) were recorded after 4 and 24 hours of access to 20% alcohol (Figure S2a).

Blood alcohol concentration (BAC) measurements Trunk blood was collected in heparinized capillary tubes from mice 30, 60, and 240 minutes after an i.p. administration of 4.0 g/kg of alcohol (20%). Serum was extracted with 3.4% trichloroacetic acid followed by a 5-minute centrifugation at 420×g and assayed for alcohol content using the NAD+-NADH enzyme spectrophotometric method[6,7]. BACs were determined using a standard calibration curve.

Intermittent access to 0.03% saccharin 2-bottle choice in mice Mice were acclimated for 2 weeks to the reverse dark cycle housing conditions and then had concurrent access to one bottle of 0.03% saccharin and one bottle of water for 24 hours following the schedule described above for 3 weeks. Saccharin intake (ml/kg of body weight) and the preference ratio for the saccharin solution (volume of saccharin solution intake/total volume of fluid intake) were recorded after 4 and 24 hours of access (Figure S2a).

Intermittent limited-access to 20% alcohol, drinking in the dark (DID) procedure The procedure used here was conducted as described in[8]. Mice were acclimatized for 2 weeks to the reverse dark cycle housing conditions and then had access to a solution of 20% alcohol for 4 hours (Mondays, Wednesdays and Fridays), with access beginning 2 hours after the lights turned off (12:00 PM), for 3 weeks before the beginning of experiments (Figure S2b-c). Water was always available except during the 4-hour access to alcohol. The amount of alcohol consumed was measured as the difference in volume at the beginning and at the end of the limited-access session and expressed in g/kg of body weight/4 hours.

Intermittent limited-access to 0.03% saccharin, drinking in the dark (DID) procedure Mice received intermittent access to a saccharin solution (0.03%; w/v), following the same schedule as described above for 2 weeks before the beginning of experiments (Figure S2b). The amount of saccharin solution intake (ml/kg body of weight/4 hours) was measured as described above.

Alcohol operant self-administration The procedure was conducted as previously described[9,10]. Before the beginning of the self-administration training, rats had a 24-hour access to one bottle of 20% alcohol and one bottle of water every other day for 8 weeks, with all drinking sessions starting at 12:00 PM. The placement (left or right) of each solution was alternated between each session to control for side preference. This procedure leads to an escalation in the level of alcohol intake reaching an average of ~5-6 g/kg/24 hours which generates BACs of ~81 mg%[11]. Only rats consuming more than 4 g/kg/24 hours were used in the alcohol operant self-administration procedure[9]. Rats were then trained to self-administer a 20% alcohol solution during a 30-minute session, 5 days a week (one session per day) for 3 months before the

beginning of experiments (Table S2, Figure S2d). During each session, rats had access to 2 levers: 3 presses on the active lever resulted in one alcohol delivery [0.1 ml of 20% alcohol; fixed ratio 3 (FR3) reinforcement schedule]. Presses on the inactive lever were recorded but no programmed events occurred. Only rats pressing for more than 0.4 g/kg/30 minutes at baseline were used in the study[9]. To assess alcohol-seeking behavior, rats were tested in a single extinction session trial (30 minutes), in which pressing on the active lever (i.e., the alcohol-associated lever) did not result in alcohol delivery.

Sucrose operant self-administration The sucrose operant self-administration procedure was conducted as previously described in[8]. Briefly, rats were trained to self-administer a solution of 1.5% of sucrose (w/v) 5 days/week during a 30-minute session (one session per day), for 3 months before the beginning of the experiment (Table S3, Figure S2d). During each session, rats had access to 2 levers: 3 presses on the active lever resulted in one sucrose solution delivery (0.1 ml of 1.5% sucrose solution) and presses on the inactive lever were recorded but no programmed events occurred. To measure sucrose-seeking behavior, rats were tested in a single extinction session trial (30 minutes) in which pressing the active lever (i.e., sucrose-associated lever) did not result in sucrose delivery.

Biochemical analyses Mice were killed by cervical dislocation. Rats were anesthetized by isoflurane and were killed by decapitation. Brains were rapidly removed and placed on an ice-cold platform. Nucleus accumbens (NAc) were dissected and immediately homogenized in ice-cold radio immunoprecipitation assay (RIPA) buffer (in mM: 50 Tris-HCl, 5 EDTA, 120 NaCl, and 1% NP-40, 0.1% deoxycholate, 0.5% SDS, protease and phosphatase inhibitor cocktail

inhibitors). Samples were then briefly sonicated using a sonic dismembrator, and protein content was quantified using a BCA™ protein assay kit. Samples were resolved on NuPAGE 4-12% Bis-Tris gradient gels at 120 V for 2-3 hours. After a 2-hour transfer onto nitrocellulose membranes, blots were blocked in 5% milk/TBST for one hour and probed with a pan-acetylated lysine H4 antibody (1:1000), anti-Dnmt1 (1:1000), or anti-GAPDH (1:5000) antibodies. Membranes were then washed and probed with horseradish peroxidase-conjugated secondary antibodies. Membranes were then stripped for 30 minutes at room temperature in a buffer containing 25 mM Glycine-HCL, 1% (w/v) SDS, pH 3.0, and reprobed with the anti-H4 antibody (1:1000). Immunoreactivity was detected using ECL. The optical density of the relevant immunoreactive band was quantified using the NIH ImageJ 1.45s program.

Data analysis Depending on the experiment, data were analyzed with unpaired or paired *t*-test, one-way ANOVA or two-way ANOVA with or without repeated measures (RM), as indicated. Significant main effects and interactions of the ANOVAs were further investigated using Student-Newman-Keuls post hoc analysis. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SEM.

References

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