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RESEARCH ARTICLE

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Systemic depletion of histone macroH2A1.1 boosts hippocampal synaptic plasticity and social behavior in mice

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Abstract

Gene expression and epigenetic processes in several brain regions regulate physiological processes such as cognitive functions and social behavior. MacroH2A1.1 is a ubiquitous variant of histone H2A that regulates cell stemness and differentiation in various organs. Whether macroH2A1.1 has a modulatory role in emotional behavior is unknown. Here, we employed macroH2A1.1 knock-out ($^{-/-}$) mice to perform a comprehensive battery of behavioral tests, and an assessment of hippocampal synaptic plasticity (long-term potentiation) accompanied by whole hippocampus RNA sequencing. MacroH2A1.1$^{-/-}$ mice exhibit a stunningly enhancement both of sociability and of active stress-coping behavior, reflected by the increased social behavior in social activity tests and higher mobility time in the forced swim test, respectively. They also display an increased hippocampal synaptic plasticity, accompanied by significant neurotransmission transcriptional networks changes. These results suggest that systemic depletion of histone macroH2A1.1 supports an epigenetic control necessary for hippocampal function and social behavior.

KEYWORDS

epigenetics, hippocampus, histone macroH2A1.1, social behavior

Abbreviations: CNS, central nervous system; CS, conditioned and innocuous stimulus; EPM, elevated plus maze; FC, fear conditioning; FST, forced swim test; IPA, ingenuity pathway analysis; KO, knockout; LTP, long-term potentiation; NOR, novel object recognition; OF, open field; PPI, prepulse inhibition; Sinv, social investigation test; SIT, social interaction test; US, unconditioned and aversive stimulus.

Vincenzo Micale and Manlio Vinciguerra authors are Co-senior authorship.

1 | INTRODUCTION

In addition to canonical histones (H1, H2A, H2B, H3, and H4), histone variants replace replication-coupled histones in a subset of nucleosomes, conferring chromatin unique properties to modulate gene expression.[1] Histone variants have specific genomic distribution; they are regulated by ad hoc deposition and removal machineries and have important roles in development, cell plasticity and cancer.[1] MacroH2A1 is a variant of histone H2A, coded by the gene *H2AF*Y, that regulates cell plasticity and proliferation during pluripotency and tumorigenesis,[2,3] and participates in the formation of senescence-associated heterochromatic foci (SAHF) in senescent cells.[2] Moreover, macroH2A1transcript levels have been found to be upregulated in the brains of patients and in mouse models of Huntington's disease, a neurodegenerative disease characterized by movement disorders, psychiatric symptoms and dementia,[4] and in Alzheimer's disease.[5] Interestingly, Zovkic et al highlighted histone variant exchange (eg, H2A.Z) as a mechanism contributing to the molecular basis of cognitive function and memory.[6] Since macroH2A functions as a chromatin component and transcriptional regulator,[7] and defects in the mechanisms of chromatin silencing are the underlying cause for several mental retardation syndromes, including Rett syndrome and the Fragile-X syndrome,[8] it would be plausible that macroH2A histone variants replacement could have a role in the development of neuropsychiatric conditions. MacroH2A1 is present in two alternatively exon-spliced isoforms, macroH2A1.1 and macroH2A1.2 and, recently, significant mRNA expression for the two isoforms has been found in the brain of adult mice[9]; however, the role of the two isoforms in the regulation of central nervous system (CNS) functions has never been investigated.

Here, we assessed the role of macroH2A1.1 in affecting emotional behavior by using whole-body knock-out ($^{-/-}$) mice for the histone macroH2A1.1.[9] Mice were submitted to a battery of behavioral tests for assessing emotional, cognitive, and social domains.[10] In addition, we evaluated hippocampal synaptic plasticity, and specifically long-term potentiation (LTP), which is particularly relevant in neuropsychiatric disorders.[11] We show here that genetic depletion of the histone macroH2A1.1 increments hippocampal synaptic plasticity at the transcriptional and functional level, and boosts social behavior. We propose that systemic deletion of macroH2A1.1 might provide a strong rationale for an epigenetic therapy aiming at improving social behavior.

2 | METHODS AND MATERIALS

2.1 | Animals

Mice lacking macroH2A1.1 were generated as follows[9]: a 12 kb segment of the murine H2AFY gene (introns 5-8) was subcloned from a BAC by recombineering into p15A-HSV tk-DTA-amp. A lacZ-neo cassette, flanked by loxP and rox sites at the 5′and 3′ ends, respectively, was inserted into the intron between exons 6a and 6b, also by recombineering. Another rox site was inserted upstream of exon 6a and another loxP site inserted downstream of exon 6b so that Dre/rox recombination[12] would remove exon 6a and the lacZ-neo cassette, and Cre/loxP recombination would remove exon 6b and the lacZ-neo cassette; thus, Cre recombination will eliminate macroH2A1.1 expression. Southern blotting of genomic NheI-digested DNA from individual ES-cell-derived clones with a 3′ probe was used to identify homologous recombinants.[9] A 12.3-kb DNA fragment corresponds to the wild-type macroH2A1.1 locus; integration of the loxP-flanked neomycin cassette 3′ of exon 6b introduced an additional NheI site, thus increasing the size of the NheI DNA fragment to 16.2 kb in the targeted allele.[9] Cre-mediated recombination resulted in a 3.9-kb NheI DNA fragment recognized by the 3′ probe, which is diagnostic of the macroH2A1.1 allele. The targeting of the macroH2A1.1 allele was performed by electroporation of A9 ES cells, which were then injected into C57BL/6 eight cell-stage embryos. The targeted macroH2A1.1$^{Fl/Fl}$ mice were crossed to delete HPRT-Cre mice (129S1/Sv-Hprttm1(CAG-cre)Mnn/J), purchased from Jackson Laboratories, USA, to remove the loxP-flanked neomycin cassette and generate macroH2A1.1$^{Fl/-}$ mice (heterozygous, HET), respectively. Mice heterozygous for the macroH2A1.1 allele were further crossed to delete Cre mice to generate the macroH2A1.1$^{-/-}$ (knockout, KO) mice, respectively. All mice used were obtained after eight generations of back crossing on a C57Bl/6 genetic background. Mice were bred and maintained at the EMBL Mouse Biology Unit, Monterotondo, or at Plaisant Srl (Rome, Italy), in accordance with current European and Italian legislation (2010/63/EU, DI 26/2014). All animal procedures were approved by the Ethical Committee (OPBA) of the Italian National Health Institute (Rome, Italy) and by the Italian Ministry of Health (specific authorization n° 183/2019-PR, protocol 6629-P-08-03-2019).

2.2 | Behavioral test and experimental design

Behavioral experiments in 6-8 weeks old mice, were conducted in battery with 3-4 days in between two consecutive tests, in the following order: (1) low- or mild-stress situation, (a) exploratory-based approach avoidance conflict tests: open field (OF)[10,13–15] elevated plus maze (EPM),[10,16,17] spontaneous alternation and spatial recognition memory in the *Y*-maze,[18] novel object recognition (NOR),[19] (b) social approach: reciprocal social interaction (SIT) and social investigation test (Sinv),[10,20] (c) sensorimotor gating in the

prepulse inhibition, PPI[21,22]; (2) high-stress situation: (d) fear conditioning (FC) test[22–25]; (e) hot plate test[26] (e) depressive-like behavior paradigm: forced swim test (FST).[17,27,28]

2.2.1 | Spontaneous locomotor activity

Exploratory activity of macroH2A1$^{-/-}$ and macroH2A1.1$^{Fl/Fl}$ mice was evaluated in the open field (OF) test, as described previously.[10,16,17] The experiment was performed in a squared box (60 × 60 × 60 cm), in which the animal was placed in the central zone of the apparatus equipped with infrared beams (TruScan; Coulbourn Instruments, Allentown, PA, USA) and allowed to explore for 20 minutes at 300 lux. Horizontal locomotion (total, margin, or central distance moved) were analyzed during the 20-min monitoring period. Each session was recorded on a HDD using a video-camera and then scored off-line by an experienced observer by means of a video/computer system ANY-MAZE. After each session, the apparatus was cleaned with a solution containing neutral soap.

2.2.2 | Elevated plus maze

The apparatus consisted of two opposite open arms, (30 × 5 cm) and two arms with walls (30 × 5 × 14 cm) that were attached to a central platform (5 × 5 cm) to form a cross. The maze was elevated 50 cm from the floor.[10,16,17] Illumination measured at the center of the maze was 300 lux. The animal was placed in the center of the maze facing one of the closed arms, and observed for 5 minutes, according to the following parameters: number of entries in the open or closed arms and time of permanence in each arm (ie, the time spent by the animal in the open or closed arms). An entry was defined as all four paws having crossed the line between an arm and the central area. It is accepted that the anxiolytic effect of a drug treatment is illustrated by increased parameters in open arms (time and/or number of entries; Ref. 16: for pharmacological validation of our current set-up). The augmented percentage of entries in open arms over the total entries in both arms is a good indicator of reduced anxious-like phenotype as well. Entries in closed arms and total entries reflect the motor component of the exploratory activity. On removal of each mouse, the maze floor was carefully wiped with a wet towel. All trials were recorded on a HDD using a video camera and then scored off-line by an experienced observer by means of a video/computer system ANY-MAZE.

2.2.3 | Three trial *Y* maze

Working and spatial recognition memory was assessed by spontaneous alternation behavior in the *Y*-maze task as described in Melnikova et al (2006) with some modifications.[29] Experiments were carried out in a *Y*-maze constructed from grey Plexiglas and consisting of three identical alleys diverging at a 120° angle one to the other and an equilateral triangular central area. During the first trial (trial 1) the mouse was placed in the center of the *Y*-maze and allowed to explore freely for 5 minutes. The sequence of arm entries was recorded. An arm entry occurs when a mouse moves all four paws into the arm crossing the threshold of the central zone. An alternation is defined as the entry in a different arm of the maze in three consecutive arm entries. The number of maximum alternations is the total number of arm entries minus two and the percentage of alternations is calculated as the ratio of actual to maximum alternations multiplied by 100: (actual alternations/maximum alternations) × 100. One week later, two more different trials (5 minutes each) were performed. In the first one (trial 2), an arm was closed allowing the mouse to explore only two arms of the maze. In trial 3, performed after 10 minutes, the arm block was removed and the mouse had free access to the three arms. Total number of arm entries, number of entries in each arm, as time spent and distance travelled by the animal was measured during all three trials.

2.2.4 | NOR test

NOR was performed as previously described with minor modifications.[19] Briefly, mice were habituated to an open field chamber (43 × 43 × 20) for 20 minutes. After 24 hours, two identical objects were placed in the maze equidistant from the walls and the center of the open field and mice were allowed to explore the two objects for10 minutes. After 1 hour during which mice came back in their home cages, the trial was repeated with one familiar and one novel object for 10 minutes. The time spent exploring the familiar and novel object was recorded using ANY-maze video tracking system (UGO BASILE). A mouse was considered to be exploring the object if it was sniffing, touching or facing it within 2 cm or less, and measurements were recorded in seconds. Between each trial, the open field was cleaned with a solution containing neutral soap to eliminate olfactory cues. To determine novelty preference, a discrimination index (DI) was calculated as follows: [(time spent with the novel object−time spent with the old object)/(time spent with the novel object + time spent with familiar object) × 100].

2.2.5 | Social interaction test

The test was carried out in a moderately illuminated room, as previously described.[10,20] After 5 minutes of habituation in a similar test cage, 2 unfamiliar mice of same age, sex, and

genotypes were placed at the opposite ends of a Plexiglas box (60 × 60 × 60 cm) and left free to interact with each other for 10 minutes. Social behaviors were defined as following, sniffing, mounting, nosing, and grooming. The arena was cleaned with ethanol (70%) and dried after each trial. The whole testing phase was recorded and analyzed by two observers blind to the genotype groups. We recorded the time spent in social behaviors and the number of interactions.

2.2.6 | Social investigation test

Social investigation (SInv) task was conducted as previously described.[30] The test took place in a Plexiglas three chamber box divided into three equal compartments (30 × 30×30 cm) that were interconnected by small opening (6 × 5 cm) with guillotine doors. An empty perforated plastic cup was placed in each side of the box. During the habituation phase the animal was placed in the center chamber of the test box and allowed to explore only this room for 5 minutes. After this session, an unfamiliar mouse of the same age, sex and genotype was placed in one of the cup. The cup in the opposite position was left empty. Both doors were opened and the animal was allowed to explore all three compartments for 10 minutes. The number of interactions with both cups as the time spent in each chamber and in direct contact with the cups, was recorded and analyzed off-line by two observers blind to the genotype groups.

2.2.7 | Forced swim test

The forced swim test (FST) employed here was essentially similar to that described elsewhere.[17,27,28] Mice were individually placed into transparent cylinders (height 23.5 cm; diameter 16.5 cm) containing 15 cm water at 25 ± 1°C for 6 minutes. The water was changed after each trial. After vigorous activity, swimming attempts cease and the animal adopts a characteristic immobile posture. A mouse is judged to be immobile when it floats in upright position and makes only small movements to keep its head above water. The duration of mobility was recorded during the last 4-min of the 6-min testing period. All trials were recorded for subsequent off-line analysis.

2.2.8 | Pre-pulse inhibition

PPI is traditionally adopted to evaluate sensorimotor gating capabilities in mammals through the analysis of the reduction in startle response produced by the presentation of a prepulse. The experimental apparatus (Med Associates inc. St Albans, VT, United States of America) was constituted by a platform with a transducer amplifier (PHM-250-60) and an acoustic stimulator (ANL-925). The apparatus was positioned in a foam-lined isolation chamber (ENV-018S). Dimmed lighting and ventilation were guaranteed by a red light and a fan positioned inside the chamber. To ensure that the experimental subjects remained on the platform, the latter was enclosed in a perforated compartment. Experimental data were acquired and analyzed through dedicated software (SOF-815). The test was carried out as previously described.[21,31] Briefly, mice were individually placed in the startle chamber and left undisturbed for 5 minutes for habituation. On the following day, mice were positioned inside the startle chamber and exposed to a continuous white noise (62 dB) for 5 minutes; following this acclimation, mice were exposed to 10 pulses of 120 dB interspaced by an average inter-trial interval (ITI) of 15 seconds. Finally, an 8-min session started, consisting of 28 trials. Each trial started with a 50-ms null period, followed by a 20-ms pre-pulse noise burst of 74, 78, 82, or 84 dB. The delay between the pre-pulse and the startle (40-ms 120 dB white noise) was 100-ms The experiment entailed the following types of trial: pre-pulse plus startle (four trials per pre-pulse intensity), prepulse alone (four trials per pre-pulse intensity), startle alone (four trials), and no stimulation (four trials). To prevent habituation, the ITI varied between 10 and 20 seconds. Galvanic response (dependent variable) was measured every millisecond for 65 ms after the onset of the startle. PPI was measured as: $PPI = [(A - B)/A] * 100$, wherein A is the baseline Galvanic reflex in response to the startle stimulus alone, and B is the reflex in response to the startle in pre-pulse plus startle trials.

2.2.9 | Fear-conditioning test

Cued fear-conditioning is typically adopted to investigate the ability of laboratory rodents to learn and remember an association between conditioned and innocuous stimulus, CS (auditory cue of 2 kHz at 86 dB, in our case) and unconditioned and aversive stimulus, US (electric foot-shock of 0.7 mA).[22–25] The apparatus consisted of a soundproof cubicle (55 × 60 × 57 cm) and a chamber (17 × 17 × 25 cm; Ugo Basile 7532, Comerio, Italy) used for test. The grid floor of the chamber (steels, 0.2 cm diameter and spaced 0.5 cm) was connected to a shock generator scrambler (Ugo Basile, conditioner 7531). The test comprised three phases: conditioning, tone, and extinction. During the conditioning phase (duration 555 seconds), test mice were individually positioned into the test chamber and, following a 180 seconds acclimation period, were exposed to three CS, each of 30 seconds. During the last 2 seconds of each CS, mice received a US. Each CS was separated from the following by 95 seconds of ITI without presentation of either tone or shock. After 24 hours, mice were tested for contextual memory (context test, duration

300 seconds) in the same chamber where conditioning was performed without CS and US presentations. The tone test (duration 360 seconds) was conducted 2 hours after the context test in a novel chamber (with different properties than those of the conditioning chamber). After the first 180s of acclimation, a CS (the conditioning tone) was presented for the last 180 seconds. Duration of freezing was acquired through dedicated software (ANY-maze, Stoelting Co.) during the tone and extinction phases as an index of emotional memory.[22] Between sessions, the apparatus was cleaned with 30% ethanol/water solution.

2.2.10 | Hot-plate test

This test evaluates thermal pain reflexes due to footpad contact with a heated surface. We performed this test to control for the possibility that inter-individual differences in the social transmission of pain were associated to differential pain sensitivity.[26] Besides, KO and respective WT littermates were subjected to this test to assess whether macroH2A1.1 deletion could modulate pain sensitivity.[22] The test was performed at the end of the extinction phase of the cued fear-conditioning test. The apparatus consisted of a metal plate 25 × 25 cm (Socrel Mod. DS-37, Ugo Basile, Italy) over which a transparent Plexiglas cylinder (20 cm diameter; 40 cm height) was placed. During the test, when the metal plate was heated to 52 ± 0.5°C, each mouse was individually placed within the Plexiglas cylinder, onto the metal plate and the latency to the first hind paw-licking and/or front paw-licking was measured. The test was terminated if the latency exceeded the cut-off time of 60 seconds.[22] The metal plate and Plexiglas cylinder were cleaned with 30% ethanol/water solution. The test was performed under dim lights and it was video-recorded (Sony DCR-SX21E) to allow for subsequent accurate determination of latencies.

The order of tests within the battery was designed in such manner that mice would be evaluated on what were thought to be least invasive tests before being tested on more invasive assays. This design was developed with the assumptions that testing from least to most invasive would allow for recovery time between tests and would reduce the likelihood that behavioral responses would be influenced by previous testing experience. Two weeks after the end of the behavioral tests, animals were killed and the brains used for hippocampal slice preparation and evaluation of synaptic plasticity.

2.3 | Hippocampal slice electrophysiology

Synaptic transmission and LTP in hippocampal slices were evaluated as previously described,[32] and detailed in Figure 2A. To evaluate synaptic transmission and LTP in hippocampal slices, 8- to 10-week-old macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice were killed by decapitation, the brains isolated and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing, in mM, 126 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$ and 11 glucose, pH 7.3, saturated with 95% O_2 and 5% CO_2. The brains were then sectioned by using a vibratome to obtain parasagittal slices (400 μm) containing the hippocampus. Slices were allowed to recover for at least 1 hour at room temperature in ACSF, and then a single slice was transferred to a submerged recording chamber and continuously superfused with ACSF (2.7-3 mL/min, 32-33°C).[33] Field excitatory post-synaptic potentials (fEPSPs) were recorded in the *stratum radiatum* of CA1 area of the hippocampus with a glass microelectrode filled with 2 M NaCl (1-3 MΩ resistance) after stimulation of the Schaffer collaterals through an insulated bipolar twisted NiCr electrode and a S48 Square Pulse Stimulator (Grass Instruments) (Figure 2A). Traces were acquired, amplified and analyzed with DAM-80 AC differential amplifier (WPI Instruments) and with the LTP software.[34] Stimuli (100 μsec duration, delivered every 20 seconds) were set to an intensity that evoked half-maximal fEPSPs, and three consecutive fEPSPs were averaged. LTP was induced by high frequency stimulation protocol (HFS) consisting in 2 trains of 100 Hz, with 10 seconds interval between each train. Synaptic transmission was recorded for 70 minutes and 10 minutes of stable baseline recordings preceded HFS; early phase LTP (E-LTP) was quantified in the last 10 minutes of recording and expressed as percentage change with respect to the average slope of the fEPSP measured during the 10 minutes of baseline. Slices that did not exhibit stable fEPSP slopes during the first 10 minutes of recording were excluded from the study. For experiments in young mice, we used 14 and 15 slices obtained from 5 macroH2A1.1$^{Fl/Fl}$ and 5 macroH2A1.1$^{-/-}$mice, respectively. For experiments in 12-month-old mice, 10 slices obtained from 4 mice were used for each genotype.

2.4 | RNA-sequencing and bioinformatics analyses

For RNA-Seq, total RNA was extracted from whole hippocampi isolated from macroH2A1$^{Fl/Fl}$(controls) and macroH2A1.1$^{-/-}$ mice, analyzed as described previously.[9] Indexed libraries were prepared from 2 mg/ea purified RNA with the TruSeq Total Stranded RNA Sample Prep Kit (Illumina, Cambridge, UK) according to the manufacturer's instructions. Libraries were quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) and pooled so that each index-tagged sample was present in equimolar amounts; the final concentration of the pooled samples was 2 nmol/L. Pooled samples were then subjected to cluster generation and sequencing using an Illumina HiSeq

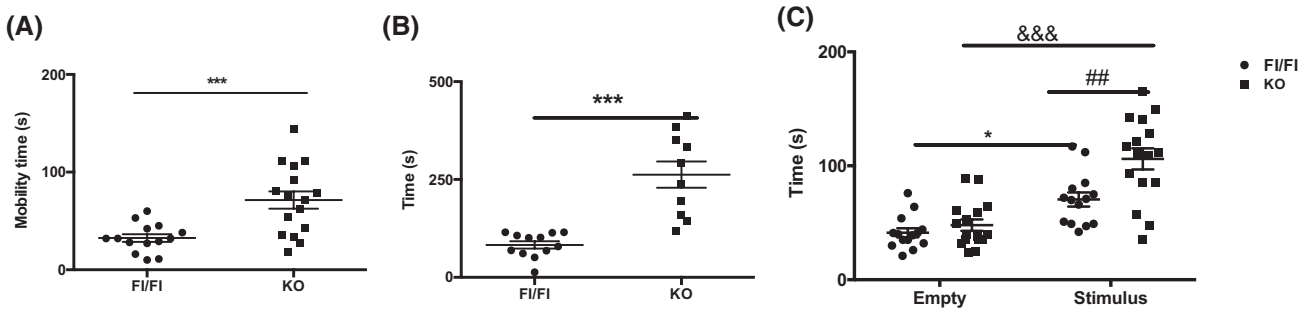

FIGURE 1 Behavioral characterization of macroH2A1.1$^{-/-}$ knockout (KO) mice and their respective control littermates (macroH2A1.1$^{Fl/Fl}$). A, Forced swim test. Data are presented as mean ± SEM (n = 14-16 mice/group) regarding the mobility time expressed in seconds. B, Social interaction test. Data are presented as mean ± SEM (n = 10-12 pairs/group) of time of interaction expressed in seconds. C, Social investigation test. Data are presented as mean ± SEM (n = 14-16 mice/group) of time of interaction expressed in seconds.*$P < .05$,***$P < .001$, $^{\#\#}P < .01$, or $^{\&\&\&}P < .001$ Tuckey *post-hoc* or unpaired *t*-test

2500 System (Illumina, Cambridge, UK) in a 2 × 100 paired-end format at a final concentration of 8 pmol/L.

Short reads were aligned against the GRCm38 genome assembly using STAR (ver. 2.5.1a). Piled up reads were counted with htseq-count. Normalization of reads counts and their comparisons were performed using the edge R R package. Genes were considered differentially expressed between groups if their expression values differed by more than 2-folds, significantly (q-value ≤ .05). Pathway enrichment analysis was performed by using Ingenuity Pathway Analysis (QIAGEN Inc). All computations were performed with R ver. 3.4.1 (R Core Team 2017).

2.5 | Statistical analyses

Data were analyzed using two-or three-way ANOVA, followed by Tukey post-hoc, if appropriate. Student's *t* test or the non-parametric Mann-Whitney *U* test, was used to analyze independent data macroH2A1.1$^{-/-}$*vs* macroH2A1$^{Fl/Fl}$. Statistical evaluations were performed using specialized software (Graph-Pad Prism 6.0). Data are shown as means ± standard error of the mean (SEM) and statistical significance was accepted at $P \leq .05$.

3 | RESULTS

3.1 | MacroH2A1.1$^{-/-}$ mice exhibit an increased sociability and active stress coping behavior

Genetic deletion of macroH2A1.1 did not affect exploratory activity of mice in the OF test, as compared to macroH2A1.1$^{Fl/Fl}$ (control) littermates (Total Distance: $t = 0.1042$, $P = .9178$; Central Distance: $t = 0.1544$, $P = .8784$; Figure S1). In the EPM test, an exploration-based paradigm for innate anxiety, macroH2A1.1 deletion did not affect the exploration of the aversive open arms in terms of time spent in open arms (TOA: $t = 0.07495$, $P = .9408$), number of entries in open arms (EOA: $t = 0.05692$, $P = .5738$), and total number of arm entries (TAE: $t = 0.04778$, $P = .6365$), as parameters to assess anxiety-like behavior and locomotor activity, respectively (Figure S2). Although a potential role of macroH2A in neurological disease exists,[35] deletion of histone macroH2A1.1 did not affect the cognitive performance since no difference was found in spontaneous alternation of the *Y*-maze test ($t = 1.926$, $P = .0656$; Figure S3), in the discrimination index of NOR ($t = 1.262$; $P = .2220$; Figure S4) as well as in the time spent among the three arms in the two trial *Y*-maze test (two-way ANOVA, arm × genotype interaction $F_{2,78} = 2.114$, $P = .1276$; Figure S5) between macroH2A1.1$^{-/-}$ and macroH2A1.1$^{Fl/Fl}$ animals. Both macroH2A1.1$^{-/-}$ and macroH2A1.1$^{Fl/Fl}$ mice acquired a clear freezing response during FC training (two-way ANOVA, factor inter-trial interval: $F_{2,52} = 34.403$, $P < .001$) exhibiting an increased freezing duration over the course of footshock presentation (Figure S6A). No difference between the two genotype was found in the freezing response to the context (two-way ANOVA, factor genotype: $F_{1,26} = 0.27$, $P = .87$; Figure S6B) or to the tone presentation (two-way ANOVA, factor genotype: $F_{1,26} = 1.58$, $P = .21$; Figure S6B) over the entire observation periods. When we analyzed in 1-min intervals, all mice showed the same freezing response (Figure S6C,D). Freezing before tone presentation (baseline) was low and similar between the two groups (Figure S6B). To evaluate whether individual differences in FC responses may be explained by differential basal pain sensitivity, KO and WT mice were tested in the hot-plate paradigm. Both groups of mice showed indistinguishable pain sensitivity expressed as the first latency (in sec) to paw licking (KO: 28.38 seconds, WT: 25.33 seconds, MWU-test = 37, $P = .0792$).

There was an increase in arousal of macroH2A1.1$^{-/-}$ mice, as revealed in the acoustic startle paradigm ($F_{1,26} = 8.315$, $P = 0.007$; Figure S7A). Both macroH2A1.1$^{-/-}$ and macroH2A1.1$^{Fl/Fl}$ mice showed similar PPI levels (two-way

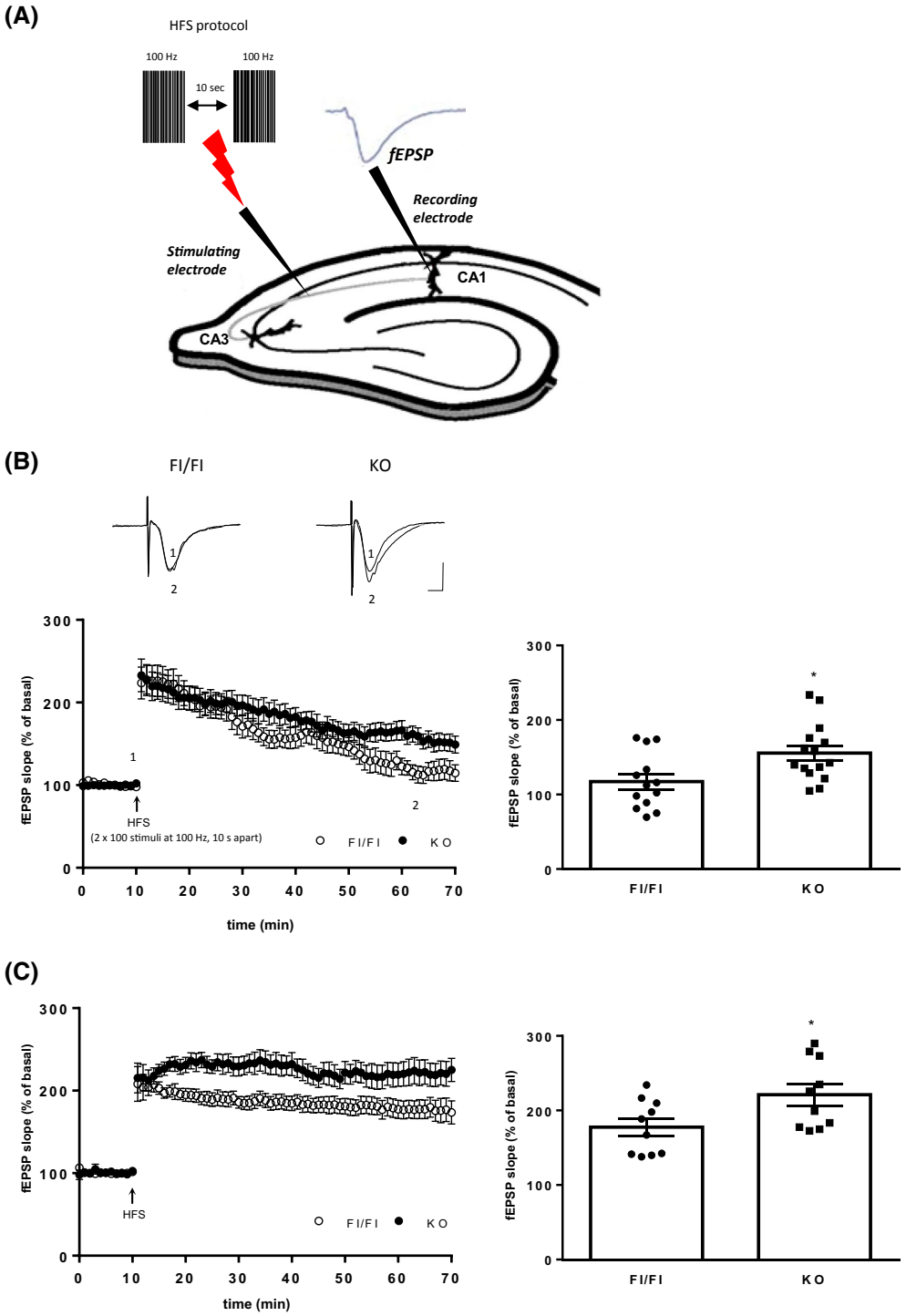

FIGURE 2 Long-term potentiation (LTP) in macroH2A1.1$^{Fl/Fl}$ and in macroH2A1.1$^{-/-}$ mice. A, Field EPSPs (fEPSPs) were recorded in the CA1 area of hippocampal slices; LTP was induced by HFS of Schaffer collaterals consisting in two trains of 100 Hz with 10 s interval between each train. B, Left panel: time courses of fEPSP slope after HFS in 8- to 10-week-old macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice; insets show fEPSP recorded in basal condition (1) and 60 minutes after HFS (2). Right panel: bar graph showing potentiation of fEPSP in macroH2A1.1$^{Fl/Fl}$ (n = 14) and macroH2A1.1$^{-/-}$ (n = 15) mice, 60 minutes after HFS (*$P < .05$ *vs*macroH2A1.1$^{Fl/Fl}$, unpaired Student *t*-test). C, Left panel: time courses of fEPSP slope after HFS in 12-month-old macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice. Right panel: bar graph showing fEPSP potentiation in macroH2A1.1$^{Fl/Fl}$ (n = 10) and macroH2A1.1$^{-/-}$ (n = 10) mice, 60 minutes after HFS (*$P < .05$ *vs* macroH2A1.1$^{Fl/Fl}$, unpaired Student *t*-test)

ANOVA, factor genotype $F_{1,26} = 0.171, P = .89$) that increased according to prepulse intensity ($F_{3,78} = 18.562$, $P < .001$), with 74 dB eliciting the lowest response compared to 78, 82 and 84 dB (Figure S7B). No gender difference was detected in any of the above behavioral performances (*data not shown*). MacroH2A1.1$^{-/-}$ mice showed a significant increase

of mobility time in the FST as index of enhanced responsiveness to a stressful situation resulting in increased active coping behavior ($t = 3.822$, $P < .001$, Figure 1A). The above behavioral performance was not gender dependent since male and female macroH2A1.1$^{-/-}$ showed the same phenotype (two-way ANOVA, factor genotype: $F_{1,26} = 12.93$, $P = .0013$; factor gender: $F_{1,26} = 0.08584$, $P = .7719$; genotype × gender interaction: $F_{1,26} = 0.0073$, $P = .9326$; Figure S8A). In the SIT, as compared to the macroH2A1.1$^{Fl/Fl}$ animals, macroH2A1.1$^{-/-}$ showed a better behavioral performance, as described by the significant increase in time of interaction ($t = 5.595$, $P < .001$; Figure 1B). Interestingly, female macroH2A1.1$^{-/-}$ mice spent more time in the reciprocal social interaction than male macroH2A1.1$^{-/-}$ mice ($P < .05$), suggesting that deletion of macroH2A1.1 elicited a gender related effect in the improved social activity in the SIT (two-way ANOVA, factor genotype: $F_{1,18} = 50.83$, $P < .001$; factor gender: $F_{1,18} = 3.558$, $P = .0755$; genotype × gender interaction $F_{1,18} = 9.454$, $P = .0065$; Figure S8B). In the Sinv test although macroH2A1.1$^{-/-}$ ($P < .001$) and macroH2A1.1$^{Fl/Fl}$ ($P < .05$) mice have higher interest for the tube containing the mouse, genetic deletion of macroH2A1.1 improved the social performance of mice since macroH2A1.1$^{-/-}$ mice spent more time than macroH2A1.1$^{Fl/Fl}$ ($P < .001$) to interact with the social stimulus (two-way ANOVA: factor genotype: $F_{1,18} = 50.83$, $P < .001$; factor stimulus: $F_{1,18} = 3.558$, $P = .0755$; genotype × animal interaction $F_{1,18} = 9.454$, $P = .0065$; Figure 1C). This effect was not gender dependent (three way-ANOVA: factor gender: $F_{1,52} = 1.355$, $P = .2497$; genotype × stimulus × gender interaction $F_{1,52} = 0.06777$, $P = .7956$; Figure S8C). Interestingly, the better social activity cannot be explained by changes in locomotor activity (Figure S9A,B).

3.2 | Early-phase LTP in macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice

Several studies suggested that the hippocampal function is implicated in mice performance in SIT and FST,[36,37] which we found improved in macroH2A1.1$^{-/-}$ mice. To unravel whether macroH2A1.1 histone variant may impact hippocampal synaptic transmission and synaptic plasticity, we recorded extracellular fEPSPs in the CA1 area of hippocampal slices after stimulation of Schaffer collaterals in macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice (Figure 2A).

To evaluate synaptic plasticity, we delivered HFS to the Schaffer collaterals, a protocol known to induce LTP of the synaptic transmission in CA1 neurons.[38] In our experiments, we considered the magnitude of synaptic potentiation 60 minutes after HFS, thus evaluating the early phase of LTP (E-LTP). In macroH2A1.1$^{Fl/Fl}$ mice HFS induced a prompt increase of fEPSP slope that, however, slowly declined 60 minutes after HFS (117.3% ± 10.35%) (Figure 2B). On the contrary, in macroH2A1.1$^{-/-}$ HFS of the Schaffer collaterals resulted in a long-lasting significant increase of fEPSP slope (155.5% ± 9.97%, 60 minutes after HFS) with respect to basal values, which was statistically different from that of macroH2A1.1$^{Fl/Fl}$ ($P = .013$ Student's t test) (Figure 2B). Since the magnitude of LTP increases with age,[39] and in order to observe E-LTP in macroH2A1.1$^{Fl/Fl}$we repeated the experiments in hippocampal slices from 12 month-old animals. In this case, we found, 60 minutes after HFS of the Schaffer collaterals, a robust E-LTP in the pyramidal neurons of the CA1 area not only in macroH2A1.1$^{-/-}$ mice (221% ± 14.65% of the basal values) but also in macroH2A1.1$^{Fl/Fl}$(177.6% ± 11.53% of the basal values). Also in this case, however, macroH2A1.1$^{-/-}$ mice show a significant increase in the magnitude of E-LTP with respect to macroH2A1.1$^{Fl/Fl}$ ($P = .031$, Student t-test, Figure 2C).

No gender difference was observed between the two genotypes. Of note, basal synaptic transmission, evaluated with input/output curves and paired pulse facilitation ratio, was not different between macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice (Figure S10). Our data describe a new macroH2A1.1-dependent action in the hippocampus, defined as a facilitation of long-term potentiation.

3.3 | RNA-sequencing from hippocampi of macroH2A1.1 KO mice uncovers altered transcriptional signaling networks

To gain a mechanistic insight into hippocampal functions associated with decreased macroH2A1.1 expression, which could play a role in the phenotype of macroH2A1.1$^{-/-}$, we performed transcriptome-wide RNA sequencing (RNA-Seq) to identify differentially expressed genes (DEGs) between hippocampi isolated from macroH2A1.1$^{-/-}$ and macroH2A1.1$^{Fl/Fl}$ male and female mice. Heatmaps drawn for the commonly DEGs between the two genotypes revealed a strikingly different profile, if both genders were pooled together, or if only males or if only females were considered separately (Figure 3A). We identified 301 overall (in males + females), 423 (in males), 291 (in females) DEGs, of which 143 overall (in males + females), 123 (in males), 162 (in females) were upregulated and 158 overall (in males + females), 129 (in females), 300 (in males) were downregulated in macroH2A1.1$^{-/-}$ compared to macroH2A1.1$^{Fl/Fl}$ controls (Figure 3B). When considering both genders, an unbiased Ingenuity Pathway Analysis (IPA) revealed that these genes significantly over-represented the adrenomedullin signaling pathway (GUCY2C,MYLK3,TFAP2B,TFAP2D), GABA receptor signaling (GABRA6,SLC6A12), and stem cell pluripotency (SALL4, WNT9B), as 3 top significant pathways (Table 1) (Figure 4A). When considering the genders

separately, the genes belonging to these pathways were still over-represented, (Figure 4B,C). In addition, Wnt/β-catenin and stem cell pluripotency signaling pathways were over-represented in both genders (Figure 4B,C).

4 | DISCUSSION

Here, we described for the first time the role of histone variant macroH2A1.1, a splicing isoform of the ubiquitous large histone macroH2A1, in the regulation of emotional behavior in mice in highly selective manner. Behavior is the result of a multitude of physiological and cognitive processes that have to be coordinated and fine-tuned within the organism. Thus, behavioral performance appeared to be a suitable read-out to test whether global deletion of macroH2A1.1 histone leaves a detectable behavioral phenotype. To this purpose we used an ad hoc knock-out (macroH2A1.1$^{-/-}$) mouse model, which we recently characterized for macroH2A1.1′ cell autonomous contribution to the regulation of haematopoiesis.[9] Growing evidence indicates that measuring stress related conditions from different tests could reflect different states of affective behaviors. This prompted us to use different behavioral paradigms such as exploration-based tests and social paradigms, which primarily focus on reciprocal social interaction and on the preference for social stimulus, respectively, as well as tasks involving a strong mnemonic component, such as fear-based tests, to assess different aspects that could mimic symptoms of human stress-related disorders as agoraphobia, social phobia, or post-traumatic stress disorder.

The first novel result of the present study was that macroH2A1.1$^{-/-}$ mice failed to show alterations in spontaneous exploration and locomotor behavior as well as they did not show any anxiety-like phenotype when tested in exploratory behavioral paradigms such as OFT and EPM, suggesting that macroH2A1.1 histone seems to not be involved both in the

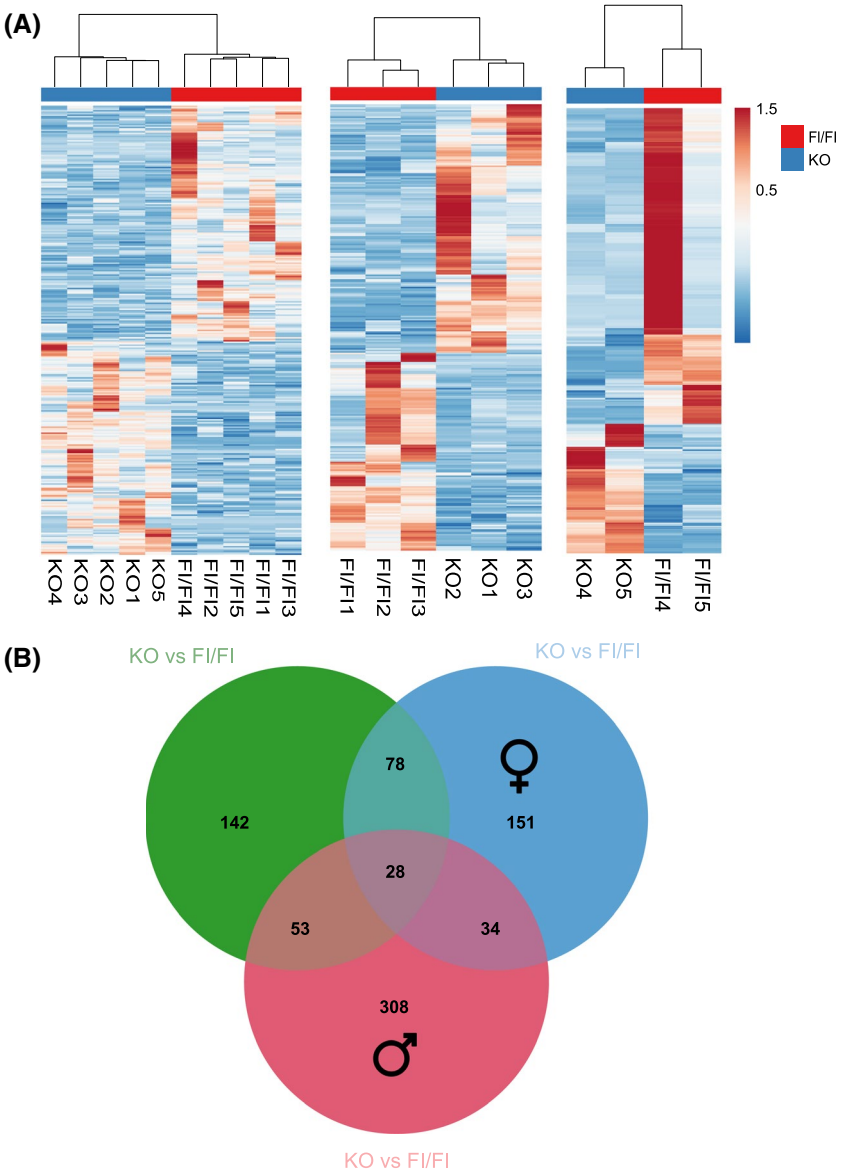

FIGURE 3 A, Heatmaps representing the expression values of differentially expressed genes between macroH2A1.1$^{-/-}$ compared to macroH2A1.1$^{FI/FI}$ controls considering the (left) whole cohort of mice, (center) only females or (right) only males. B, VENN diagram showing the level of overlap between differentially expressed genes between macroH2A1.1$^{-/-}$ compared to macroH2A1.1$^{FI/FI}$ controls considering the (green) whole cohort of mice, (pink) only females or (blue) only males

TABLE 1 Differential expression of genes overrepresented in the top three significant pathways enriched in macroH2A1.1$^{-/-}$ compared to macroH2A1.1$^{Fl/Fl}$ controls, considering the whole cohort (males + females)

Pathway	Gene	log2FC in mH2A1.1$^{-/-}$ vs mH2A1.1$^{Fl/Fl}$	*P* value
Adrenomedullin	GUCY2C	1.54	.0479
Adrenomedullin	MYLK3	2.88	9.44E−07
Adrenomedullin	TFAP2B	2.10	.0446
Adrenomedullin	TFAP2D	2.08	.0476
GABA	GABRA6	−2.52	.0481
GABA	SLC6A12	−1.62	.0007
Stemness	SALL4	−1.77	.0205
Stemness	WNT9B	1.77	.0460

locomotor activity (whose modification could affect the emotional behavior in rodents), as well as in the conflict between exploration and avoidance of a novel environment. Similarly, deletion of macroH2A1.1 did not affect spatial and working memory, as well as the extinction of aversive memories which this latter was not mediated by different basal pain sensitivity, suggesting that, although the pronounced expression of macroH2A in the prefrontal cortex[35] and in neurodegenerative disorders,[4] its deletion failed to modify the cognitive performance of mice. Among human and animal behaviors, sociability is one of the most complex behaviors taking place between conspecifics and results in social relationship, which in turn plays a role in their physiological development.[40] Social withdrawal is a highly disabling, treatment-refractory symptom of different neuropsychiatric developmental disorders such as autism and schizophrenia, whose biological basis is not fully understood. In the SIT, a test based on reciprocal social interactions it became apparent that macroH2A1.1$^{-/-}$ animals spent more time in reciprocal social interactions as the test continued, in a gender dependent manner since female KO showed better behavioral performance in terms of time of interaction with unfamiliar co-specific as compared to male macroH2A1.1$^{-/-}$.

Another frequently used test to investigate social behavior is the social investigation approach test. This test focus on the preference for social stimulus while reciprocal social interactions are intentionally avoided by allowing the test mice to explore all chambers while target mice are caged within a plastic tube.[41] In this test, both genotypes show a significant preference for a mouse over the empty tube, with a significant prevalence in the social approach of macroH2A1.1$^{-/-}$ animals ($P < 0.01 vs^{Fl/Fl}$). These findings indicate that macroH2A1.1 deletion enhances the preference for social stimulus in male and female mice and strengthens the interpretation that social behavior is not modified as a result of changes in exploratory and/or anxiety-like behavior, as confounding factors.

Immobility behavior in FST is thought to reflect either a failure of persistence in escape-directed behavior (that is, behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli.[42,43] Therefore, the increased mobility of macroH2A1.1$^{-/-}$ mice in the FST, might rather reflect enhanced responsiveness to a stressful situation resulting in increased active coping behavior.

Efficient hippocampal synaptic transmission plays a critical role by forming relational memory representations that underlies cognition and social behavior both in rodents[44] and in humans.[45] Experiences modify behavior through activity dependent, long-lasting changes of synaptic strength, which are the basis of synaptic plasticity, in particular in hippocampal CA1 and CA2 regions.[46–49] LTP is the major form of synaptic plasticity in the CNS, it is thought to play a role in the mechanisms of learning and memory and in the early development of neural circuitry,[50] and dysregulation of hippocampal LTP underlies a large number of neuropsychiatric disorders.[32,51–53] In the present study we found a significant improvement of E-LTP in the CA1 area of the hippocampus in macroH2A1.1$^{-/-}$ with respect to control mice. Actually, in young macroH2A1.1$^{Fl/Fl}$ mice HFS did not induce a long-lasting potentiation of synaptic transmission. This finding was somehow unexpected, even though differences in the induction and expression of LTP in CA1 area of hippocampal slices from 8- to 12-week-old mice have been reported among different inbred mouse strains, with C57BL/6 mice showing the highest percentage of potentiation after HFS and 129/SvEms, on the contrary, no significant potentiation.[54] Our macroH2A1.1$^{Fl/Fl}$ and macroH2A1.1$^{-/-}$ mice have been generated in a mixed C57BL/6-129S1/Sv background. Electrophysiological findings suggest that the influence of the 129S1/Sv strain is presumably preponderant with respect to C57BL/6, and stronger stimulation protocols should probably be used to induce LTP in macroH2A1.1$^{Fl/Fl}$ mice. However, the protocol we used (2 trains, 100 Hz, 10 seconds interval) allowed us to unravel facilitation in synaptic plasticity in macroH2A1.1$^{-/-}$ with respect to macroH2A1.1$^{Fl/Fl}$ mice, a difference that could be difficult to highlight with a stronger stimulation. In order to observe a long lasting potentiation of synaptic transmission also in macroH2A1.1$^{Fl/Fl}$, we repeated electrophysiology experiments in 12-month-old mice, since higher LTP magnitude was observed in older

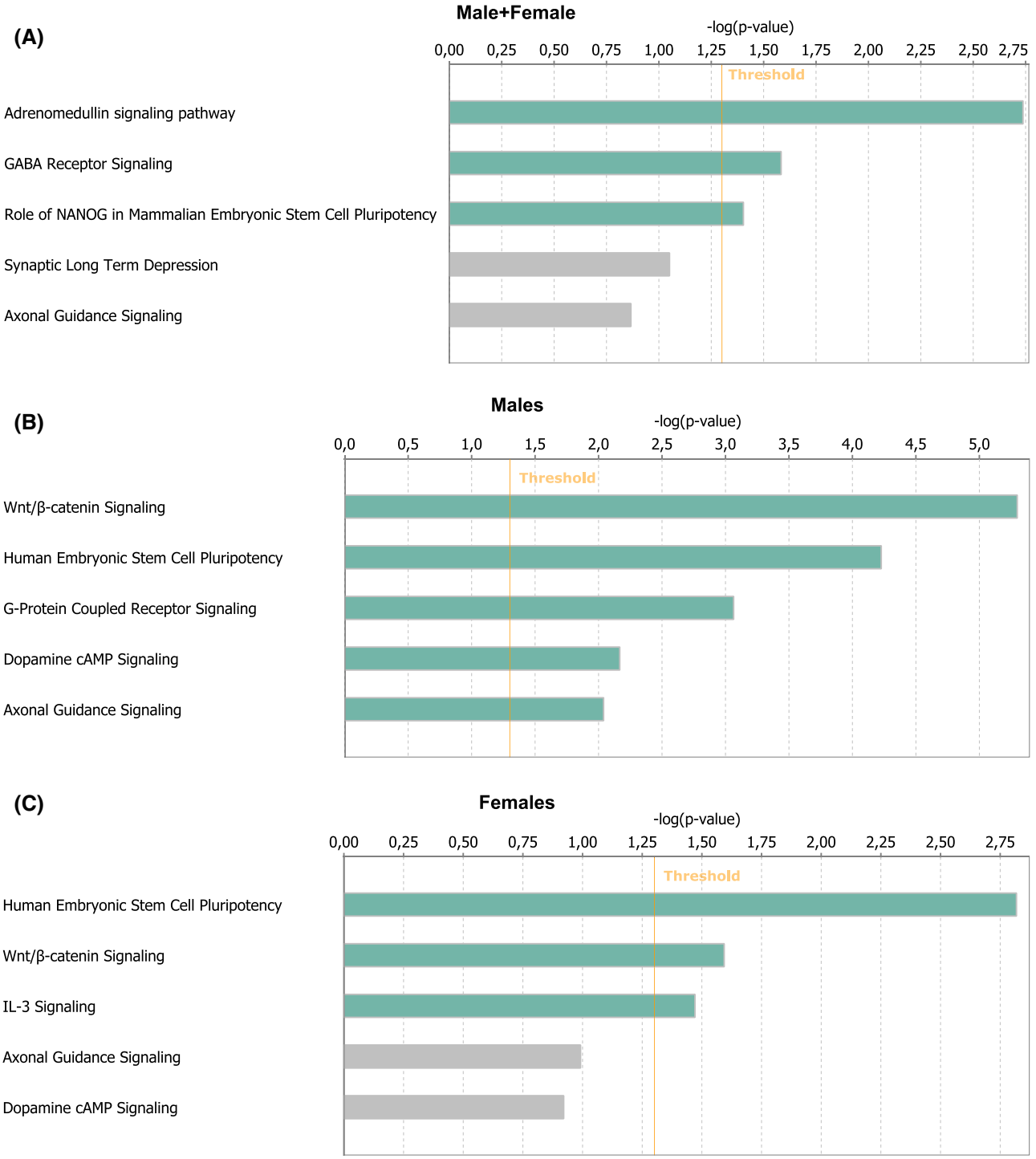

FIGURE 4 Top five significant pathways enriched by differentially expressed genes between macroH2A1.1$^{-/-}$ compared to macroH2A1.1$^{Fl/Fl}$ controls, considering (top) the whole cohort, (center) only males, or (bottom) only females

animals with respect to young.[39] In this case, we found a significant long-lasting potentiation of synaptic transmission in the CA1 area of hippocampal slices both in macroH2A1.1$^{Fl/Fl}$ and in macroH2A1.1$^{-/-}$ mice. However, the magnitude of E-LTP was significantly higher in macroH2A1.1$^{-/-}$ than in macroH2A1.1$^{Fl/Fl}$, thus further demonstrating that the depletion of histone variant macroH2A1.1 increases hippocampal E-LTP in older mice too. These results suggest that the histone variant macroH2A1.1 is strongly involved in the regulation of CA1 hippocampal neurophysiology, which, at least, could play a role in the social behaviour.

Social behaviour is a complex phenomenon based on the ability of humans and animals to properly communicate with conspecifics, whose disruption is a shared symptom of several neuropsychiatric disorders such as schizophrenia, autism, social anxiety, and it is mediated by several brain

areas (so called "social brain"), including the hippocampus.[40] Among hippocampal main sub regions that could have a role in the social activity, several data suggest that CA2 is mostly involved in integrating the complex stimuli necessary for the recognition process in the social memory.[46,55,56] The interaction with conspecific involves also the CA1 area of the ventral hippocampus, as described by Rao and colleagues[57] showing that ventral CA1 region responds to the interaction with conspecific, but not to the object presence. In our study, we did not dissect the specific involvement of hippocampal regions in the social activity of macroH2A1.1 KO mice or the role of ventral hippocampus. Thus, future studies investigating the ventral hippocampus or the CA2 area will be necessary to understand the mechanisms involved in the enhanced social activity of macroH2A1.1 KO mice.

MacroH2A1.1 did not appear to be involved in hippocampus-dependent forms of memory, but we observed changes in E-LTP. Although some data suggest that LTP changes match with analogous modifications of memory processes, many studies investigating different genetically modified mouse strains report clear dissociation of LTP and memory, challenging the causal relationship between these two processes.[58–60] Consistent with these studies, in macroH2A1.1$^{-/-}$ mice an increased hippocampal E-LTP is not associated with enhanced cognitive performance. This apparent discrepancy at least could be sought in the complexity of the processes and multitude of cerebral areas interacting each other in information storage. Our IPA analysis on the transcriptomes of isolated hippocampi from the whole cohort of macroH2A1.1$^{-/-}$ and macroH2A1.1$^{Fl/Fl}$ mice, uncovered a significant enrichment of a discrete number of DEG involved in the adrenomedullin signaling pathway, GABA receptor signalling, and stem cell pluripotency. We found increased expression of adrenomedullin pathway associated genes (GUCY2C, MYLK3, TFAP2B, TFAP2D) in the hippocampi of macroH2A1.1$^{-/-}$mice, which display increased sociability and active stress coping behavior. Consistently, behavioral analysis of mice with no adrenomedullin showed anxiety, and lower survival under stress conditions.[61] In macroH2A1.1$^{-/-}$ we observed a significant decrease in the gene expression levels of GABRA6 and the betaine-GABA transporter SLC6A12, which play complex roles in the induction of GABA-dependent long-term potentiation and long-term depression in hippocampal inhibitory synapses.[62] We identified two DEG involved in stem cell pluripotency in the hippocampi of macroH2A1.1$^{-/-}$ compared to macroH2A1.1$^{Fl/Fl}$ mice: SALL4 (significantly decreased), WNT9B (significantly increased). SALL4 is implicated in the control of the differentiation of cranial motor neurons,[63] but its role in hippocampal plasticity is unknown. WNT/β-catenin signaling is a principal regulator of adult hippocampal neurogenesis and function, and induces the proliferation and self-renewal of hippocampal stem/progenitor cells.[64] Our data cannot conclude that macroH2A1.1 hippocampal expression is sufficient for the required for the elevated LTP as well as for the observed behavioral changes: other macroH2A1.1-depleted tissues other than the brain may contribute; further studies using strains using the Cre-recombinase under the promoter of genes specifically expressed in mouse hippocampal CA1 (ie, CaMKIIα) or CA2 (ie, Amigo2-Cre) regions are warranted.

A main limitation of our study relies in its observational nature, without the identification of a specifying signalling pathway playing a major role in macroH2A1.1 dependent hippocampal and social behavioural effects. Histones have a wide genome binding distribution. Our previous deep epigenetic analyses performed in cell models of liver cancer and of adipogenesis revealed complex patterns, where the changes in genome occupancy of macroH2A1 isoforms did not correlate significantly with the induced changes in gene expression, suggesting a differential cooperation with transcription factors in altered physio-pathological states, which are difficult to dissect through an hypothesis-based approach.[65,66] Uncovering the protein interactome and the post-translational modifications of macroH2A1 isoforms in other mice models of hippocampal-dependent social behaviour, where the histones remain expressed, will likely provide more insight on the observed phenotype of macroH2A1.1$^{-/-}$ mice. Interestingly, in the cortex of the mouse line BTBR/J, a strain that shows lower sociability compared to the C57BL/6J and thus is often utilized as a model for ASD, macroH2A1 gene expression is significantly upregulated.[67]

In conclusion, our work establishes a pervasive epigenetic role for the histone variant macroH2A1.1 in the positive regulation of social behavior. Further behavioral and molecular studies are necessary to assess if and how macroH2A1 histone variants could play a role in the development of neuropsychiatric disorders.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

AUTHOR CONTRIBUTIONS

V. Chiodi, M.R. Domenici, V. Micale, and M. Vinciguerra designed research. V. Chiodi, M.R. Domenici, T. Biagini, M. Di Rosa, T. Mazza, V. Micale, and M. Vinciguerra analyzed data. V. Chiodi, M.R. Domenici, R. De Simone, A.M. Tartaglione, M. Di Rosa, O. Lo Re, V. Micale, and M. Vinciguerra performed research. V. Chiodi, M.R. Domenici Domenici, T. Biagini, T. Mazza, V. Micale, and M. Vinciguerra wrote the paper.

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REFERENCES

1. Buschbeck M, Hake SB. Variants of core histones and their roles in cell fate decisions, development and cancer. *Nat Rev Mol Cell Biol*. 2017;18:299-314.
2. Lo Re O, Vinciguerra M. Histone macroH2A1: a chromatin point of intersection between fasting, senescence and cellular regeneration. *Genes*. 2017;8(12):367.
3. Giallongo S, Rehakova D, Raffaele M, Lo Re O, Koutna I, Vinciguerra M. Redox and epigenetics in human pluripotent stem cells differentiation. *Antioxid Redox Signal*. 2021;34(4):335-349.
4. Hu Y, Chopra V, Chopra R, et al. Transcriptional modulator H2A histone family, member Y (H2AFY) marks Huntington disease activity in man and mouse. *Proc Natl Acad Sci U S A*. 2011;108(41):17141-17146.
5. Guttula SV, Allam A, Gumpeny RS. Analyzing microarray data of Alzheimer's using cluster analysis to identify the biomarker genes. *J Alzheimer's Dis*. 2012;2012:649456.
6. Zovkic IB, Paulukaitis BS, Day JJ, Etikala DM, Sweatt JD. Histone H2A.Z subunit exchange controls consolidation of recent and remote memory. *Nature*. 2014;515:582-586.
7. Perche PY, Vourc'h C, Konecny L, et al. Higher concentrations of histone macroH2A in the Barr body are correlated with higher nucleosome density. *Curr Biol*. 2000;10:1531-1534.
8. Serrano M. Epigenetic cerebellar diseases. *Handb Clin Neurol*. 2018;155:227-244.
9. Bereshchenko O, Lo Re O, Nikulenkov F, et al. Deficiency and haploinsufficiency of histone macroH2A1.1 in mice recapitulate hematopoietic defects of human myelodysplastic syndrome. *Clin Epigenetics*. 2019;11:121.
10. Terzian AL, Drago F, Wotjak CT, Micale V. The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors. *Front Behav Neurosci*. 2011;5:49.
11. Monday HR, Younts TJ, Castillo PE. Long-term plasticity of neurotransmitter release: emerging mechanisms and contributions to brain function and disease. *Annu Rev Neurosci*. 2018;41:299-322.
12. Anastassiadis K, Fu J, Patsch C, et al. Dre recombinase, like Cre, is a highly efficient site-specific recombinase in *E. coli*, mammalian cells and mice. *Dis Model Mech*. 2009;2:508-515.
13. Uttl L, Szczurowska E, Hajkova K, et al. Behavioral and pharmacokinetic profile of indole-derived synthetic cannabinoids JWH-073 and JWH-210 as compared to the phytocannabinoid Delta(9)-THC in rats. *Front Neurosci*. 2018;12:703.
14. Stark T, Ruda-Kucerova J, Iannotti FA, et al. Peripubertal cannabidiol treatment rescues behavioral and neurochemical abnormalities in the MAM model of schizophrenia. *Neuropharmacology*. 2019;146:212-221.
15. Drago F, Nicolosi A, Micale V, Lo Menzo G. Placebo affects the performance of rats in models of depression: is it a good control for behavioral experiments? *Eur Neuropsychopharmacol*. 2001;11:209-213.
16. Pamplona FA, Henes K, Micale V, Mauch CP, Takahashi RN, Wotjak CT. Prolonged fear incubation leads to generalized avoidance behavior in mice. *J Psychiatr Res*. 2011;45:354-360.
17. Direnberger S, Mues M, Micale V, et al. Biocompatibility of a genetically encoded calcium indicator in a transgenic mouse model. *Nat Commun*. 2012;3:1031.
18. Hazane F, Krebs MO, Jay TM, Le Pen G. Behavioral perturbations after prenatal neurogenesis disturbance in female rat. *Neurotox Res*. 2009;15:311-320.
19. Ferrante A, Pezzola A, Matteucci A, et al. The adenosine A2A receptor agonist T1–11 ameliorates neurovisceral symptoms and extends the lifespan of a mouse model of Niemann-Pick type C disease. *Neurobiol Dis*. 2018;110:1-11.
20. Terzian AL, Micale V, Wotjak CT. Cannabinoid receptor type 1 receptors on GABAergic vs. glutamatergic neurons differentially gate sex-dependent social interest in mice. *Eur J Neuorsci*. 2014;40:2293-2298.
21. Macri S, Ceci C, Onori MP, et al. Mice repeatedly exposed to Group-A beta-Haemolytic Streptococcus show perseverative behaviors, impaired sensorimotor gating, and immune activation in rostral diencephalon. *Sci Rep*. 2015;5:13257.
22. Zoratto F, Sbriccoli M, Martinelli A, Glennon JC, Macri S, Laviola G. Intranasal oxytocin administration promotes emotional contagion and reduces aggression in a mouse model of callousness. *Neuropharmacology*. 2018;143:250-267.
23. Yen YC, Mauch CP, Dahlhoff M, et al. Increased levels of conditioned fear and avoidance behavior coincide with changes in phosphorylation of the protein kinase B (AKT) within the amygdala in a mouse model of extremes in trait anxiety. *Neurobiol Learn Mem*. 2012;98:56-65.
24. Hofelmann D, di Benedetto B, Azad SC, Micale V, Wotjak CT, Rammes G. Lack of interaction of endocannabinoids and 5-HT(3) neurotransmission in associative fear circuits of the amygdala: evidence from electrophysiological and behavioural experiments. *Brain Res*. 2013;1527:47-56.
25. Micale V, Drago F, Noerregaard PK, Elling CE, Wotjak CT. The cannabinoid CB1 antagonist TM38837 with limited penetrance to the brain shows reduced fear-promoting effects in mice. *Front Pharmacol*. 2019;10:207.
26. Laviola G, Zoratto F, Ingiosi D, et al. Low empathy-like behaviour in male mice associates with impaired sociability, emotional memory, physiological stress reactivity and variations in neurobiological regulations. *PLoS One*. 2017;12:e0188907.
27. Murgatroyd C, Patchev AV, Wu Y, et al. Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat Neurosci*. 2009;12:1559-1566.
28. Micale V, Kucerova J, Sulcova A. Leading compounds for the validation of animal models of psychopathology. *Cell Tissue Res*. 2013;354:309-330.
29. Melnikova T, Savonenko A, Wang Q, et al. Cycloxygenase-2 activity promotes cognitive deficits but not increased amyloid burden in a model of Alzheimer's disease in a sex-dimorphic pattern. *Neuroscience*. 2006;141:1149-1162.
30. Smit-Rigter LA, Wadman WJ, van Hooft JA. Impaired social behavior in 5-HT(3A) receptor knockout mice. *Front Behav Neurosci*. 2010;4:169.
31. Zoratto F, Oddi G, Gori E, et al. Social modulation of risky decision-making in rats (*Rattus norvegicus*) and tufted capuchin monkeys (*Sapajus* spp.). *Behav Brain Res*. 2018;347:37-48.
32. De Filippis B, Chiodi V, Adriani W, et al. Long-lasting beneficial effects of central serotonin receptor 7 stimulation in female mice modeling Rett syndrome. *Front Behav Neurosci*. 2015;9:86.

33. De Filippis D, Russo A, De Stefano D, et al. Palmitoylethanolamide inhibits rMCP-5 expression by regulating MITF activation in rat chronic granulomatous inflammation. *Eur J Pharmacol*. 2014;725:64-69.
34. Anderson WW, Collingridge GL. The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. *J Neurosci Methods*. 2001;108:71-83.
35. Akbarian S, Chen RZ, Gribnau J, et al. Expression pattern of the Rett syndrome gene MeCP2 in primate prefrontal cortex. *Neurobiol Dis*. 2001;8:784-791.
36. Kalman E, Keay KA. Hippocampal volume, social interactions, and the expression of the normal repertoire of resident-intruder behavior. *Brain Behav*. 2017;7:e00775.
37. Gerlai R. Behavioral tests of hippocampal function: simple paradigms complex problems. *Behav Brain Res*. 2001;125:269-277.
38. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*. 1993;361:31-39.
39. Diogenes MJ, Costenla AR, Lopes LV, et al. Enhancement of LTP in aged rats is dependent on endogenous BDNF. *Neuropsychopharmacology*. 2011;36:1823-1836.
40. Barak B, Feng G. Neurobiology of social behavior abnormalities in autism and Williams syndrome. *Nat Neurosci*. 2016;19:647-655.
41. Sankoorikal GM, Kaercher KA, Boon CJ, Lee JK, Brodkin ES. A mouse model system for genetic analysis of sociability: C57BL/6J versus BALB/cJ inbred mouse strains. *Biol Psychiat*. 2006;59:415-423.
42. Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discovery*. 2005;4:775-790.
43. Commons KG, Cholanians AB, Babb JA, Ehlinger DG. The rodent forced swim test measures stress-coping strategy, not depression-like behavior. *ACS Chem Neurosci*. 2017;8:955-960.
44. van der Kooij MA, Sandi C. Social memories in rodents: methods, mechanisms and modulation by stress. *Neurosci Biobehav Rev*. 2012;36:1763-1772.
45. Tzakis N, Holahan MR. Social memory and the role of the hippocampal CA2 region. *Front Behav Neurosci*. 2019;13:233.
46. Hitti FL, Siegelbaum SA. The hippocampal CA2 region is essential for social memory. *Nature*. 2014;508:88-92.
47. Meira T, Leroy F, Buss EW, Oliva A, Park J, Siegelbaum SA. A hippocampal circuit linking dorsal CA2 to ventral CA1 critical for social memory dynamics. *Nat Commun*. 2018;9:4163.
48. Freund TF, Buzsaki G. Interneurons of the hippocampus. *Hippocampus*. 1996;6:347-470.
49. Okuyama T, Kitamura T, Roy DS, Itohara S, Tonegawa S. Ventral CA1 neurons store social memory. *Science*. 2016;353:1536-1541.
50. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. *Neuron*. 2004;44:5-21.
51. Lundbye CJ, Toft AKH, Banke TG. Inhibition of GluN2A NMDA receptors ameliorates synaptic plasticity deficits in the Fmr1(-/y) mouse model. *J Physiol*. 2018;596:5017-5031.
52. Glerup S, Bolcho U, Molgaard S, et al. SorCS2 is required for BDNF-dependent plasticity in the hippocampus. *Mol Psychiatry*. 2016;21:1740-1751.
53. Chalkiadaki K, Velli A, Kyriazidis E, et al. Development of the MAM model of schizophrenia in mice: sex similarities and differences of hippocampal and prefrontal cortical function. *Neuropharmacology*. 2019;144:193-207.
54. Nguyen PV, Duffy SN, Young JZ. Differential maintenance and frequency-dependent tuning of LTP at hippocampal synapses of specific strains of inbred mice. *J Neurophysiol*. 2000;84:2484-2493.
55. Alexander GM, Farris S, Pirone JR, Zheng C, Colgin LL, Dudek SM. Social and novel contexts modify hippocampal CA2 representations of space. *Nat Commun*. 2016;7:10300.
56. Oliva A, Fernandez-Ruiz A, Leroy F, Siegelbaum SA. Hippocampal CA2 sharp-wave ripples reactivate and promote social memory. *Nature*. 2020;587:264-269.
57. Rao RP, von Heimendahl M, Bahr V, Brecht M. Neuronal responses to conspecifics in the ventral CA1. *Cell Rep*. 2019;27(12):3460-3472.e3.
58. Gu Y, McIlwain KL, Weeber EJ, et al. Impaired conditioned fear and enhanced long-term potentiation in Fmr2 knock-out mice. *J Neurosci*. 2002;22:2753-2763.
59. Wegener S, Buschler A, Stempel AV, et al. Defective synapse maturation and enhanced synaptic plasticity in Shank2 Deltaex7(-/-) Mice. *eNeuro*. 2018;5.
60. Campbell SL, van Groen T, Kadish I, Smoot LHM, Bolger GB. Altered phosphorylation, electrophysiology, and behavior on attenuation of PDE4B action in hippocampus. *BMC Neurosci*. 2017;18:77.
61. Fernandez AP, Serrano J, Tessarollo L, Cuttitta F, Martinez A. Lack of adrenomedullin in the mouse brain results in behavioral changes, anxiety, and lower survival under stress conditions. *Proc Natl Acad Sci U S A*. 2008;105:12581-12586.
62. Rozov AV, Valiullina FF, Bolshakov AP. Mechanisms of long-term plasticity of hippocampal GABAergic synapses. *Biochemistry (Mosc)*. 2017;82:257-263.
63. Chilton JK, Guthrie S. Axons get ahead: Insights into axon guidance and congenital cranial dysinnervation disorders. *Dev Neurobiol*. 2017;77:861-875.
64. Lie DC, Colamarino SA, Song HJ, et al. Wnt signalling regulates adult hippocampal neurogenesis. *Nature*. 2005;437:1370-1375.
65. Borghesan M, Fusilli C, Rappa F, et al. DNA hypomethylation and histone variant macroH2A1 synergistically attenuate chemotherapy-induced senescence to promote hepatocellular carcinoma progression. *Cancer Res*. 2016;76:594-606.
66. Pazienza V, Panebianco C, Rappa F, et al. Histone macroH2A1.2 promotes metabolic health and leanness by inhibiting adipogenesis. *Epigenetics Chromatin*. 2016;9:45.
67. Mizuno S, Hirota JN, Ishii C, Iwasaki H, Sano Y, Furuichi T. Comprehensive profiling of gene expression in the cerebral cortex and striatum of BTBRTF/ArtRbrc mice compared to C57BL/6J mice. *Front Cell Neurosci*. 2020;14:595607.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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