To determine whether acetate is produced from the breakdown of alcohol contributes to dynamic acetylation of histones in the brain, we used in vivo stable-isotope labelling of protein acetylation, monitored by mass spectrometry (Fig. 1a). Acetyl groups derived from ethanol were rapidly incorporated into histone acetylation in the brain, both in the hippocampus (Fig. 1b) and in the prefrontal cortex (Extended Data Fig. 1c). The incorporation of labelled alcohol into histone acetylation was dynamic in the hippocampus, and heavy labelling decreased to baseline levels 8 h after intraperitoneal injection (Extended Data Fig. 2a, b). This rapid in vivo labelling of histone acetylation in the hippocampus was absent in mice that were injected with non-labelled alcohol (Extended Data Fig. 2d). Similar labelling occurred in the liver (Fig. 1c), which is the principal site of alcohol metabolism and expresses high levels of ACSS2\(^2\). By contrast, the abundance of labelled acetylated histones was lower in skeletal muscle (gastrocnemius muscle; Extended Data Figs. 1d, 2e), which has relatively lower ACSS2 expression levels\(^3\).

To test whether ACSS2 is required for the incorporation of alcohol-derived acetate into the brain, we attenuated the expression of ACSS2 in the dorsal hippocampus (dHPC) by shRNA knockdown using a previously validated viral vector\(^2\). In these ACSS2-knockdown mice, we compared alcohol-derived histone acetylation separately in the dHPC, in which ACSS2 was reduced, and in the ventral hippocampus (vHPC), in which ACSS2 expression was not affected. Notably, knockdown of ACSS2 prevented the incorporation of heavy-labelled acetyl groups derived from alcohol into histone acetylation in the dHPC (Fig. 2a). By contrast, in the same mouse, incorporation of the heavy label into histone acetylation in the vHPC was not affected (Fig. 2b). These in vivo data indicate that acetate that is derived from the metabolism of alcohol in the liver is transported to the brain and readily incorporated into ACSS2-dependent histone acetylation (see Supplementary Information for further discussion).

Although the majority of alcohol metabolism takes place in the liver, alcohol fractions may also be converted to acetate in the brain\(^6\) by the enzymes catalase and cytochrome P450 2E1 (CYP2E1). We therefore assessed the contribution of acetyl groups that are derived from extra-cellular acetate to histone acetylation in the brain. In mice that were intraperitoneally injected with 2 g kg\(^{-1}\) deuterated acetate (acetate-d3), the labelled acetate was rapidly incorporated into histone acetylation in the brain, at similar levels in both hippocampus and cortex (Extended Data Fig. 2f, g). Relative levels of the labelled acetate were highest at 30 min and returned to background levels at 4 h after injection, indicating that acetate-derived acetyl groups were quickly incorporated into brain histone acetylation and showing the rapid turnover of this process. Notably, we found that levels of acetate in the hippocampus

Emerging evidence suggests that epigenetic regulation is dependent on metabolic state, and implicates specific metabolic factors in neural functions that drive behaviour\(^1\). In neurons, acetylation of histones relies on the metabolite acetyl-CoA, which is produced from acetate by chromatin-bound acetyl-CoA synthetase 2 (ACSS2)\(^2\). Notably, the breakdown of alcohol in the liver leads to a rapid increase in levels of blood acetate\(^3\), and alcohol is therefore a major source of acetate in the body. Histone acetylation in neurons may thus be under the influence of acetate that is derived from alcohol\(^4\), with potential effects on alcohol-induced gene expression in the brain, and on behaviour\(^5\). Here, using in vivo stable-isotope labelling in mice, we show that the metabolism of alcohol contributes to rapid acetylation of histones in the brain, and that this occurs in part through the direct deposition of acetyl groups that are derived from alcohol onto histones in an ACSS2-dependent manner. A similar direct deposition was observed when mice were injected with heavy-labelled acetate in vivo. In a pregnant mouse, exposure to labelled alcohol resulted in the incorporation of labelled acetyl groups into gestating fetal brains. In isolated primary hippocampal neurons ex vivo, extracellular acetate induced transcriptional programs related to learning and memory, which were sensitive to ACSS2 inhibition. We show that alcohol-related associative learning requires ACSS2 in vivo. These findings suggest that there is a direct link between alcohol metabolism and gene regulation, through the ACSS2-dependent acetylation of histones in the brain.
were significantly increased 30 min after injection of alcohol or acetate (Extended Data Fig. 2h), and we detected substantial amounts of heavy acetate in the hippocampus as early as 30 min after injection with deuterated ethanol (ethanol-d6) (Extended Data Fig. 3a).

We further investigated whether alcohol-derived carbon groups are incorporated into other key metabolites in hippocampal tissue. Although we detected no incorporation of the alcohol-derived label into pools of glucose and 3-hydroxybutyrate, and only a fraction (less than 1%) into the pool of lactate, we found that alcohol labels pools of glutamine in the hippocampus (Extended Data Fig. 3b–e). The in vivo synthesis of glutamine in astrocytes replenishes the glutamate–glutamine cycle, in which glutamine is trafficked into glutamatergic neurons for production of the neurotransmitter glutamate. Citrate—the substrate that is used by ATP citrate lyase to produce nucleo-cytoplasmic acetyl-CoA—is generated from α-ketoglutarate, which can be derived from carboxylation of glutamine; this path could therefore provide another route through which alcohol contributes to histone acetylation. However, we detected only traces of the alcohol-derived label in pools of citrate or isocitrate in the hippocampus (Extended Data Fig. 3f). Taken together with our mass spectrometry data in ACSS2-knockdown mice (Fig. 2a, b), these results support the view that alcohol-derived acetate that contributes to the acetylation of histones in the hippocampus is converted to acetyl-CoA directly by ACSS2. Accordingly, our data suggest that the increased levels of blood acetate from alcohol metabolism promote ACSS2-mediated dynamic acetylation of histones in the brain.

We examined the functional relevance of alcohol-derived acetate for ACSS2-dependent histone acetylation in regulating the expression of genes in the hippocampus. We found that treating wild-type mice with alcohol resulted in significant enrichment of peaks for K9-acetylated histone H3 (H3K9ac) and H3K27ac—both at key neuronal genes and genome-wide—and that this enrichment was greatly attenuated in ACSS2-knockdown mice (Fig. 2c–g; chromatin immunoprecipitation followed by sequencing (ChIP–seq) performed 1 h after alcohol injection). For example, we observed ACSS2-dependent and alcohol-induced histone acetylation at Fstl1 (follistatin-like 1) (Fig. 2c), a neuronal gene that has been implicated in the development and migration of neurons14, and alcohol-induced enrichment of H3K27ac at Cep152 (centrosomal protein of 152 kDa) (Extended Data Fig. 4a), an important regulator of genome integrity that is recurrently mutated in intellectual developmental disorders and microcephaly15. Another example is Uimc1 (ubiquitin-interaction-motif-containing 1) (Extended Data Fig. 4b), which has previously been linked with neurodevelopmental disorders and autism16. Evaluating the ChIP–seq data for histone acetylation on a genome-wide scale, we found that 74% of the H3K9ac peaks that changed after exposure to alcohol were increased (339 out of 458 changed peaks called with MACS2; 10% false discovery rate (FDR) used as the significance threshold for DiffBind; Fig. 2d), and that 60% of the differential H3K27ac peaks were increased by alcohol (490 out of 816 peaks; ChIP–seq performed 1 h after alcohol injection; Fig. 2e). Notably, this response was eliminated in ACSS2-knockdown mice: 98% of the H3K9ac and H3K27ac peaks that increased in wild-type mice after alcohol treatment were not induced in the dHPC of ACSS2-knockdown mice (Fig. 2f, g). We then performed RNA sequencing (RNA-seq) to characterize the transcriptional response and found that H3K9ac and H3K27ac drove gene expression genome-wide in wild-type mice that were treated with alcohol (Extended Data Fig. 5a, b). However, in line with the ChIP–seq data, this response was blunted in ACSS2-knockdown mice (Extended Data Fig. 5c, d). A functional analysis of the genes that were both hyperacetylated and induced by alcohol in an ACSS2-dependent manner revealed enrichment of genes with functions in protein binding, cell junctions, postsynaptic density and drug response (Extended Data Fig. 5e, f). Together, our in vivo findings show that treatment with alcohol leads to increased histone acetylation and transcriptional activity in the dHPC in an ACSS2-dependent manner.

Because alcohol and acetate have pleiotropic effects on brain circuitry and metabolism17, we developed an ex vivo assay to more closely model the direct effects of exogenous acetate on gene expression. We used isolated mouse primary hippocampal neurons, cultured for one week after isolation and subsequently treated with 5 mM acetate, to investigate the transcriptional response to supraphysiological levels of acetate that mimic the influx of exogenous acetate during alcohol intake. Furthermore, to determine the specific role of ACSS2 in transcriptional responses to acetate, we used a highly specific small-molecule inhibitor22 of ACSS2 (ACSS2i; C19H18N4O2S2) (Extended Data Fig. 6a).

In primary hippocampal neurons, supplementation with acetate induced the expression of 3,613 genes (Fig. 3a, Extended Data Fig. 6b). Using an analysis of Gene Ontology (GO) terms, we found that these genes are involved in nervous system processes, including signal transduction and learning and memory (Extended Data Fig. 6c). By contrast, treatment with acetate resulted in the downregulation of genes that are involved in immune system processes (Extended Data Fig. 6d). In the presence of ACSS2i, 2,107 of the genes that were induced by acetate were no longer upregulated (Extended Data Fig. 6f), indicating that acetate-induced transcription relies heavily on the catalytic activity of ACSS2. Notably, acetate-induced genes were not regulated by treatment with ACSS2i in the absence of acetate (Extended Data Fig. 6e). GO analysis of the upregulated genes that were sensitive to ACSS2i showed enrichment for nervous system processes, behaviour, learning and memory (Extended Data Fig. 6d) and specific genes showed sensitivity to ACSS2i (Extended Data Fig. 7a–d). For example, Slc17a7 was upregulated after treatment of wild-type hippocampus cells with acetate, but induction was diminished when ACSS2 was inhibited (Extended Data Fig. 7a). Slc17a7 encodes vesicular glutamate transporter 1, which is implicated in hippocampal synaptic plasticity, addiction and alcohol use23. In addition, impaired DNA methylation of Ccncyl (cyclin J-like) has been linked to prenatal alcohol exposure and fetal alcohol spectrum disorder (FASD)24 (Extended Data Fig. 7b). Further analysis revealed that the ACSS2i-sensitive and acetate-upregulated genes were also bound by hippocampal ACSS2 (which has been investigated by ChIP–seq previously25), and binding was proximal to the promoter at baseline without any direct behavioural stimulation in vivo (Extended Data Fig. 8a). GO analysis linked these ACSS2 target genes to intricate plasticity-related mechanisms that involve axonogenesis and the activity of voltage-gated ion channels (Fig. 3b). Correspondingly, motif analysis of ACSS2-targeted, acetate-induced and
ACSS2i-sensitive genes implicated the involvement of neuronal transcription factors—including E2F3 and NR5A2 (Fig. 3c), which have been linked to neurodifferentiation and drug-related regulation of behaviour.21,22

There was a substantial overlap between genes that were upregulated by alcohol in vivo in the dHPC and genes that were induced by acetate ex vivo—RNA-seq identified 830 alcohol-responsive hippocampal genes that overlapped with the ex vivo differentially expressed genes (Fig. 3d)—which suggests that translating our ex vivo model to the in vivo situation is valid. GO analysis for these overlapping genes highlighted the enrichment of genes that are related to neuronal plasticity, including those with roles in synapses, neuron projection and axons; however, genes related to ribosomal and mitochondrial functions were also enriched (Extended Data Fig. 8b). Notably, a previously published microarray dataset of hippocampal genes that are regulated by alcohol in vivo also showed substantial overlap with our list of genes that are induced by acetate ex vivo (81 of 214 (38%) alcohol-responsive hippocampal genes in the microarray).23 Next, we showed (starting from a complementary analysis of our in vivo data) that target genes of ACSS2 in the hippocampus that show alcohol-induced H3K9ac in vivo were also upregulated by treatment of hippocampal neurons with acetate ex vivo, and that ACSS2i blocks this gene induction (Fig. 3e). The equivalent relationship existed for hippocampal genes that show alcohol-induced H3K27ac in vivo; these genes were not induced by acetate ex vivo in the presence of ACSS2i (Fig. 3f).

Together, these findings suggest that ACSS2 may have a role in alcohol-related learning by coordinating alcohol-induced histone acetylation and gene expression. To examine potential behavioural effects in wild-type and ACSS2-knockdown mice, we performed ethanol-mediated conditioned place preference (CPP), which has previously been used to assess ethanol-associated learning.24 In this paradigm, mice are exposed to neutral and rewarding stimuli in distinct spatial compartments, distinguished by environmental cues. After conditioning, CPP is measured to neutral and rewarding stimuli in distinct spatial compartments, distinguished by environmental cues. After conditioning, CPP is measured in the conditioned and unconditioned chamber (Fig. 4b). We found that...
wild-type mice spent longer in the compartment to which ethanol was delivered during training (P = 0.0391; Wilcoxon test) (Fig. 4b). Notably, acquisition of CPP depends on spatial memory formation in the dHPC and, accordingly, dHPC lesions disrupt place conditioning. To test the importance of ACSS2 in the dHPC, we injected mice with AAV9 virus expressing shRNA against Acss2 to reduce the protein level of ACSS2 (n = 10), or control virus expressing GFP only (n = 8) (Extended Data Fig. 9a, b). We detected a significant main effect of the conditioning subgroup (P = 0.0227, F1,32 = 5.73; main effect of training from a two-way analysis of variance (ANOVA) across the four groups), showing that the ethanol-induced CPP procedure was successful. In addition, we observed a significant interaction between the ACSS2-knockdown and conditioning subgroups (P = 0.0462, F1,32 = 4.303; interaction from two-way ANOVA across the four groups), indicating that the knockdown of ACSS2 in the dHPC significantly reduced the expression of CPP. Strikingly, we found that ethanol-associated CPP was suppressed in mice in which ACSS2 expression was reduced in the dHPC (P = 0.4316; Wilcoxon test) (Fig. 4b), indicating that the formation of ethanol-related associative memories requires ACSS2.

Overall, our ex vivo and in vivo molecular data, together with our behavioural findings, show that ACSS2 is required for the incorporation of heavy-labelled acetate into acetylated histones in the dHPC, and that this facilitates memory-related gene expression and alcohol-related associative learning (Fig. 4c). These results establish ACSS2 as a promising candidate for therapeutic intervention in alcohol-use disorders, and, accordingly, dHPC lesions disrupt place conditioning. Here, we show that exposure to alcohol gives rise to the acetylation of histones in the brain both directly (through the direct incorporation of alcohol-derived acetate) and indirectly (through other metabolic pathways). Incorporation of alcohol-derived acetate into histone acetylation was recently observed in the liver. However, to our knowledge, our data provide the first empirical evidence indicating that a portion of acetate that is derived from the metabolism of alcohol directly influences epigenetic regulation in the brain. We show that this direct pathway has important functional and behavioural consequences, shedding light on a neurobiological aspect of alcohol use. Given that effects of ethanol on the brain and behaviour are complex, further studies will be required to determine the relative contributions of ethanol-derived histone acetylation, ethanol-induced intracellular signalling pathways and ethanol-related redox stress. We also show that histone acetylation in the brain occurs through the generation of acetyl-CoA by ACSS2. In the hippocampus, the incorporation of acetyl groups that are derived from alcohol may be critical for alcohol-associated associative learning, which encodes environmental cues associated with alcohol that drive craving, seeking and consumption even after protracted periods of abstinence. The direct pathway that we identify here substantially furthers our understanding of alcohol-induced epigenetic regulation in the brain, which has previously been limited to the indirect effects of alcohol-induced intracellular signalling and changes in the expression or activity of histone-modifying enzymes. The direct pathway contributes to a large proportion of the histone acetylation that occurs after ethanol exposure, and suggests that the incorporation of...
alcohol-derived acetyl groups is physiologically relevant and associated with the transcriptional and behavioural adaptations that are induced by ethanol. Notably, our findings suggest that other peripheral sources of physiological acetate—primarily the gut microbiome—may affect central histone acetylation and brain function in a similar manner, which may either control or foster other metabolic syndromes. Translational treatment strategies that target this nexus between peripheral metabolic activity and neuroepigenetic regulation may pave the way for therapeutic interventions for alcohol use and other neuropsychiatric disorders.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1700-7.

1. Li, X., Egervari, G., Wang, Y., Berger, S. L. & Lu, Z. Regulation of chromatin and gene expression by metabolic enzymes and metabolites. *Nat. Rev. Mol. Cell Biol.* 19, 563–578 (2018).
2. Mews, P. et al. Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. *Nature* 546, 381–386 (2017).
3. Sarkola, T., Iles, M. R., Kohlenberg-Mueller, K. & Eriksson, C. J. P. Ethanol, acetaldehyde, acetate, and lactate levels after alcohol intake in white men and women: effect of 4-methylpyrazole. *Alcohol. Clin. Exp. Res.* 26, 239–245 (2002).
4. Soliman, M. L. & Rosenberger, T. A. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. *Mol. Cell. Biochem.* 352, 173–180 (2011).
5. Pandey, S. C., Kyzar, E. J. & Zhang, H. Epigenetic basis of the dark side of alcohol addiction. *Neuropsycharmacology* 122, 74–84 (2017).
6. Mews, P. & Berger, S. L. in Methods in Enzymology Vol. 574 (ed. Marmorstein, R.) 311–329 (Elsevier, 2016).
7. Comerford, S. A. et al. Acetate dependence of tumors. *Cell* 159, 1591–1602 (2014).
8. Zakhari, S. Alcohol metabolism and epigenetic changes. *Alcohol Res.* 35, 6–16 (2013).
9. Bonthius, P. J. et al. Noncanonical genomic imprinting effects in offspring. *Cell Rep.* 12, 979–991 (2015).
10. Zimatin, S. M., Pronko, S. P., Vasiou, V., Gonzalez, F. J. & Detrich, R. A. Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505 (2006).
11. Liu, R. et al. Fstl1 is involved in the regulation of radial glial scaffold development. *Mol. Brain* 8, 53 (2015).
12. Kalay, E. et al. CEP152 is a genome maintenance protein disrupted in Seckel syndrome. *Nat. Genet.* 43, 23–26 (2011).
13. Stessman, H. A. F. et al. Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. *Nat. Genet.* 49, 515–526 (2017).
14. Volkow, N. D. et al. Acute alcohol intoxication decreases glucose metabolism but increases acetate uptake in the human brain. *Neuroimage* 64, 277–283 (2013).
15. Rao, P. S. S., Bell, R. L., Engleman, E. A. & Sari, Y. Targeting glutamate uptake to treat alcohol use disorders. *Front. Neurosci.* 9, 144 (2015).
16. Laufer, B. I. et al. Associative DNA methylation changes in children with prenatal alcohol exposure. *Epigenomics* 7, 1259–1274 (2015).
17. Cates, H. M. et al. Transcription factor E2F3a in nucleous accumbens affects cocaine action via transcription and alternative splicing. *Biol. Psychiatry* 84, 167–179 (2018).
18. Stergiopoulos, A. & Politis, P. K. Nuclear receptor NR5A2 controls neural stem cell fate decisions during development. *Nat. Commun.* 7, 12230 (2016).
19. Mulligan, M. K. et al. Molecular profiles of drinking alcohol to intoxication in C57BL/6J mice. *Alcohol. Clin. Exp. Res.* 35, 659–670 (2011).
20. Juarez, B. et al. Midbrain circuit regulation of individual alcohol drinking behaviors in mice. *Nat. Commun.* 8, 2220 (2017).
21. Ferbinteau, J. & McDonald, R. J. Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus* 11, 187–200 (2001).
22. Vezzey, K. J., Parnell, S. E., Miranda, R. C. & Golding, M. C. Dose-dependent alcohol-induced alterations in chromatin structure persist beyond the window of exposure and correlate with fetal alcohol syndrome birth defects. *Epigenetics Chromatin* 8, 39 (2015).
23. Mead, E. A. & Sarkar, D. K. Fetal alcohol spectrum disorders and their transmission through genetic and epigenetic mechanisms. *Front. Genet.* 5, 154 (2014).
24. Mandal, C., Halder, D., Jung, K. H. & Chai, Y. G. In utero alcohol exposure and the maternal drinking. *Front. Genet.* 7, 1100–1108 (2017).
25. Mews, P. & Calipari, E. S. in Progress in Brain Research Vol. 235 (eds Calvey, T. & Daniels, W.) 19–63 (Elsevier, 2017).
26. Egervari, G., Cicciocorpo, R., Jentsch, J. D. & Hurd, Y. L. Shaping vulnerability to addiction -the contribution of behavior, neural circuits and molecular mechanisms. *Neurosci. Biobehav. Rev.* 85, 117–125 (2018).
27. Kriss, C. L. et al. In vivo metabolic tracing demonstrates the site-specific contribution of maternal drinking: an epigenetic phenomenon of maternal drinking. *Int. J. Biol. Sci.* 13, 1100–1108 (2017).
28. Mews, P. & Calipari, E. S. in Progress in Brain Research Vol. 235 (eds Calvey, T. & Daniels, W.) 19–63 (Elsevier, 2017).
29. Cates, H. M. et al. Transcription factor E2F3a in nucleous accumbens affects cocaine action via transcription and alternative splicing. *Biol. Psychiatry* 84, 167–179 (2018).
30. Stergiopoulos, A. & Politis, P. K. Nuclear receptor NR5A2 controls neural stem cell fate decisions during development. *Nat. Commun.* 7, 12230 (2016).
31. Mulligan, M. K. et al. Molecular profiles of drinking alcohol to intoxication in C57BL/6J mice. *Alcohol. Clin. Exp. Res.* 35, 659–670 (2011).
32. Juarez, B. et al. Midbrain circuit regulation of individual alcohol drinking behaviors in mice. *Nat. Commun.* 8, 2220 (2017).
33. Ferbinteau, J. & McDonald, R. J. Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus* 11, 187–200 (2001).
34. Vezzey, K. J., Parnell, S. E., Miranda, R. C. & Golding, M. C. Dose-dependent alcohol-induced alterations in chromatin structure persist beyond the window of exposure and correlate with fetal alcohol syndrome birth defects. *Epigenetics Chromatin* 8, 39 (2015).
35. Mead, E. A. & Sarkar, D. K. Fetal alcohol spectrum disorders and their transmission through genetic and epigenetic mechanisms. *Front. Genet.* 5, 154 (2014).
36. Mandal, C., Halder, D., Jung, K. H. & Chai, Y. G. In utero alcohol exposure and the alteration of histone marks in the developing fetus: an epigenetic phenomenon of maternal drinking. *Int. J. Biol. Sci.* 13, 1100–1108 (2017).
37. Mews, P. & Calipari, E. S. in Progress in Brain Research Vol. 235 (eds Calvey, T. & Daniels, W.) 19–63 (Elsevier, 2017).
38. Egervari, G., Cicciocorpo, R., Jentsch, J. D. & Hurd, Y. L. Shaping vulnerability to addiction -the contribution of behavior, neural circuits and molecular mechanisms. *Neurosci. Biobehav. Rev.* 85, 117–125 (2018).
39. Kriss, C. L. et al. In vivo metabolic tracing demonstrates the site-specific contribution of maternal drinking: an epigenetic phenomenon of maternal drinking. *Int. J. Biol. Sci.* 13, 1100–1108 (2017).
40. Mews, P. & Calipari, E. S. in Progress in Brain Research Vol. 235 (eds Calvey, T. & Daniels, W.) 19–63 (Elsevier, 2017).
Methods

Data reporting
No statistical methods were used to predetermine sample size. All animals were randomly allocated into experimental and control groups. Mass spectrometry analysis of heavy labelled samples and analysis of behavioural experiments was performed by blinded investigators.

Stable isotope labelling of brain histone acetylation
We injected mice intraperitoneally with 2 g kg⁻¹ ethanol-d₆ or control saline, and assessed deuterium in incorporation into acetylated histones at baseline, as well as at 1 and 4 h after intraperitoneal injections (Fig. 1a). We confirmed that the injected ethanol-d₆ is readily metabolized to acetate that becomes systemically accessible (Extended Data Fig. 1a), resulting in rapid labelling of blood acetate (Extended Data Fig. 1b). Using quantitative liquid chromatography–mass spectrometry (LC–MS) we quantified the relative abundance of isotopically labelled histone acetylation in the brain and in peripheral tissues (Fig. 1a).

Analysis of the patterns of heavy labelling showed that injection of heavy-labelled ethanol leads to marked increases of the M+3 species (Extended Data Fig. 1e, f; Supplementary Table 1), which suggests that ethanol-derived acetate contributes to histone acetylation through direct deination of triply deuterated acetyl-CoA. This increase in the M+3 species was also evident when accounting for the natural levels of heavy isotopes of carbon and hydrogen (Extended Data Fig. 1g; see also Methods for detailed descriptions). The increases of M+1 and M+2 species (Extended Data Fig. 1h) could be driven by deuterion back exchange or by singly and doubly deuterated metabolites, indicating that alternative metabolic pathways also contribute to histone acetylation labelling; however, the major increase is to the M+3 species through triply deuterated acetate. We also performed tracing of ¹³C-labelled alcohol and found rapid incorporation into histone acetylation in the hippocampus, equivalent to ethanol-d₆ (Extended Data Fig. 2c). We examined whether alcohol exposure during gestation influences histone acetylation in the developing fetal brain by measuring the direct deination of alcohol-derived acetyl groups onto histones. Using the protocol described above (intraperitoneal injection of heavy-labelled alcohol (2 g kg⁻¹) followed by mass spectrometry on isolated histone proteins) we confirmed incorporation of alcohol metabolites (4 h after injection) into the acetylation of histones in neurons of gestating female mice (Fig. 4d), consistent with the previous results in males (Fig. 1b).

Histone extraction
Histones were extracted as previously described. The cells were incubated in nuclear isolation buffer (15 mM Tris–HCl, 15 mM NaCl, 60 mM KCl, 5 mM MgCl₂, 1 mM CaCl₂, and 250 mM sucrose at pH 7.5; 0.5 mM AEBSF, 10 mM sodium butyrate, 5 mM microcystin and 1 mM DTT added fresh) with 0.2% NP-40 on ice for 5 min. The nuclei were collected by centrifuging at 700 × g at 4 °C for 5 min, and the supernatant was retained. Next, histones were extracted by trypsinization of nuclear pellet with 0.2% NP-40 for 3 h at 4 °C with rotation. The insoluble nuclear debris was pelleted at 3,400 × g at 4 °C for 5 min, and the supernatant was retained. Next, histone proteins were precipitated by adding 100% trichloroacetic acid in a 1:3 ratio (v/v) for 1 h at 4 °C. The pellet was washed with acetone to remove residual acid. Histones were resuspended in 30 μl of 50 mM NH₄HCO₃ (pH 8.0).

Histone propionylation and digestion
Histones were derivatized and digested as previously described. The sample was mixed with 15 μl derivatization mix, consisting of propionic anhydride and acetonitrile in a 1:3 ratio (v/v), and this was immediately followed by the addition of 7.5 μl ammonium hydroxide to maintain pH 8.0. The sample was incubated for 15 min at 37 °C, dried and the derivatization procedure was repeated one more time. Samples were then resuspended in 50 mM NH₄HCO₃, and incubated with trypsin (enzyme:sample ratio of 1:20) overnight at room temperature. After digestion, the derivatization reaction was performed again twice to derivatize the N termini of the peptides. Samples were desalted using C₁₈ stage tips before LC–MS analysis.

Nanoscale liquid chromatography–tandem mass spectrometry
Samples were analysed by using a nanoscale liquid chromatography–tandem mass spectrometry (LC–MS/MS) setup. Nanoscale liquid chromatography was configured with a 75 μm ID × 25 cm Reprosil-Pur C₁₈-AQ nano-column (3 μm; Dr. Maisch HPLC GmbH) using an EASY-nLC nano-flow high-performance liquid chromatography (HPLC) system (Thermo Fisher Scientific), packed in-house. The HPLC gradient was as follows: 5% to 32% solvent B in solvent A (A = 0.1% formic acid; B = 80% acetonitrile, 0.1% formic acid) over 45 min, from 32% to 90% solvent B in 5 min and 90% B for 10 min at a flow rate of 300 nL min⁻¹. Nanoscale liquid chromatography was coupled to an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific). The acquisition method was data-independent acquisition (DIA) as described. In brief, two full-scan mass spectra (m/z 200–1,100) were acquired in the ion trap within a DIA duty cycle, and 16 MS/MS were performed with an isolation window of 50 Da. Normalized collision energy was set to 35%.

Data analysis
Raw mass spectrometry data were analysed manually. We selected the seven most-intense peptides of histones H3 and H4 that contained acetylations, and extracted the relative abundance of the M+1, M+2 and M+3 signals compared to the monoisotopic signal. The other peptides were not considered as, owing to their low abundance, we could not reliably quantify the relative abundance of all the isotopes. The percentage represented in the radar plots indicates the relative intensity of the M+3 signal (the fourth isotopic species) compared to the monoisotopic signal. Data were not normalized to the non-labelled sample, so that the relative abundance of the natural isotopic distribution could also be observed in the untreated mice.

For statistical analysis, we performed two-tailed heteroscedastic t-tests (significant at P < 0.05) when comparing the same isotope in treated versus untreated samples (Supplementary Table 1). In this analysis, we considered differences in the relative abundance of all species (M+1, M+2, M+3), and found that major changes occur in M+3 (Supplementary Tables 1, 2). We used the R package envlpAt to estimate the theoretical relative abundance of the first four isotopes of the seven peptides considered in this study, which showed no significant difference to the observed isotopic distribution of the untreated samples (Extended Data Fig. 1g). Natural abundance corrections were performed using FluxFix, to calculate the exact relative abundance of the M+1, M+2 and M+3 species in the samples that were treated with labelled ethanol or acetate (Extended Data Fig. 1h). We used a matrix-based approach to correct natural abundance, as proposed previously. This calculation corrects for the contribution of each isotopologue, removing, for example, from the M+3 signal, the portion of the isotopic pattern that is contributed by the increase of the M+1 and M+2 species.

ChIP–seq
ChIP-seq was performed as previously described with modifications for the preparation of mouse brains. Briefly, approximately 20 mg of dHPC from each mouse was minced on ice, cross-linked with 1% formaldehyde for 10 min and quenched with 125 mM glycine for 5 min. Nuclei were prepared by dounce homogenization of cross-linked tissue in nuclei isolation buffer (50 mM Tris–HCl at pH 7.5, 25 mM KCl, 5 mM MgCl₂, 0.25 M sucrose) with freshly added protease inhibitors and sodium butyrate. Nuclei were lysed in nuclei lysis buffer (10 mM
Tris-HCl at pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate, 0.5% N-lauroylsarcosine) with freshly added protease inhibitors and sodium butyrate, and chromatin was sheared using a Covaris S220 sonicator to approximately 250 bp in size. Equal aliquots of sonicated chromatin were used per immunoprecipitation reaction with 5 μl H3K9ac antibody (Active Motif; 39137; 09811002) or 4 μl H3K27ac antibody (Abcam; 4729; GR32313-2) preconjugated to Protein G Dynabeads (Life Technologies). Ten percent of the chromatin was saved as input DNA. ChIP reactions were incubated overnight at 4 °C with rotation and washed three times in wash buffer. Immunoprecipitated DNA was eluted from the beads, reversed cross-linked and purified together with the input DNA. Exactly 10 ng DNA (either ChIP or input) was used to construct sequencing libraries using the NEBNext Ultra II DNA library preparation kit for Illumina (New England Biolabs; NEB). Libraries were multiplexed using NEBNext Multiplex Oligos for Illumina (dual index primers) and single-end sequenced (75 bp) on the NextSeq 500 platform (Illumina) in accordance with the manufacturer’s protocol.

ChIP–seq analysis
ChIP–seq tags generated with the NextSeq 500 platform were demultiplexed with the bc2fastq utility and aligned to the mouse reference genome (assembly GRCm38 (mm10)) using Bowtie v.1.1.1, allowing up to two mismatches per sequencing tag. Peaks were detected using MACS2 (tag size = 75 bp; FDR < 1 × 10 −5) from pooled H3K9ac or H3K27ac tags of mice from the same condition along with treatment-matched input tags as control. The multiple transcription-factor-binding loci (MTL) method was used to compare H3K9ac or H3K27ac enrichment in the four study conditions. The statistical significance of differential H3K9ac or H3K27ac enrichments was assessed by using DiffBind (Biocgregator v.3.7) in a two-way comparison (wild-type mice treated with saline versus wild-type mice treated with ethanol, or ACSS2−/− mice treated with ethanol versus ACSS2−/− mice treated with ethanol). UCSC genome-browser track views were created for ChIP–seq data by first pooling replicates and generating coverage maps using BEDtools genomeCoverageBed -bg, then adjusting for library size using the RPM (reads per million) coefficient. The input control signal was then subtracted from the ChIP signal. The resulting tracks were converted from bedGraph to bigWig files using the bedGraphToBigWig function in the UCSC genome browser. RNA-seq tracks were created similarly, first splitting by tag orientation and generating coverage maps using BEDtools genomeCoverageBed -bg, then adjusting for library size using the RPM (reads per million) coefficient. The resulting tracks were converted to bigWig files, as for ChIP−seq tracks, and the + and − tags from a given sample were plotted as overlays in a track hub.

RNA-seq
All RNA-seq data were prepared for analysis as follows. NextSeq sequencing data were demultiplexed using native applications on BaseSpace. Demultiplexed FASTQs were aligned by STAR v.2.5.2 to the assembly mm10 (GRCm38). Aligned reads were mapped to genomic features using HTSeq v.0.6.1. Quantification, library size adjustment and analysis of differential gene expression was done using DESeq2 and Wald’s test (pairwise comparisons between treatment with acetate and DMSO in the inhibitor-treated or untreated cells, followed by a set overlap of differentially expressed genes). Overlaps between lists of genes were tested for significance using a hypergeometric test.

Functional analysis
GO analysis was performed using DAVID with FDR cut-off of 0.01, filtering categories with fewer than 10 genes or less than 2.5× fold enrichment over background. Motif analysis was performed using HOMER v.4.6 on all ACSS2 peaks from published in vivo data, targeting (by the nearest transcription start site) a gene sensitive to acetate with H3K9ac at the promoter using a fixed window of 300 bp.

Primary hippocampal neurons
Plated primary hippocampal neurons were obtained from the Neurons R Us neuronal core at the University of Pennsylvania. Cells were maintained in neurobasal medium (NBM; Gibco) supplemented with GlutaMAX (Gibco) and B27 (Gibco). After 7 days of differentiation, cells were treated for 24 h with 5 mM acetate or vehicle (NBM) in the presence or absence of 20 μM ACSS2i or vehicle (DMSO diluted into NBM). Cells were lysed using QIAzol (Qiagen) and RNA was extracted using the RNeasy Mini Kit (Qiagen). RNA-seq libraries were prepared using the NEBNext Ultra II Directional RNA library preparation kit (NEB).

Mouse experiments
Animal use, surgical procedures and all experiments performed were approved by the Institutional Animal Care and Use Committee (protocol 804849). All personnel involved have been adequately trained and are qualified according to the Animal Welfare Act and the Public Health Service policy. Adult male mice (8 weeks old) or E18.5 pregnant females were used. Ethanol, ethanol−d6 (Sigma-Aldrich; 186414), ethanol-1-13C (Sigma-Aldrich; 324523) and sodium acetate-3-13C (Sigma-Aldrich) were administered by intraperitoneal injection and dosed at 2 g kg −1 (20% solution in saline, filtered through a Stericup GV filter). CPP was performed as described previously. In brief, mice CPP boxes (Ugo Basile; 42533) with external dimensions 35 × 18 × 29 cm were used. The apparatus was divided into two chambers (16 × 15 × 25 cm) that differed in wall and floor pattern. Striped walls were paired with circle cutouts (1 cm) and solid grey walls were paired with square cutouts (0.5 cm). Sessions were run in a dark room at ambient temperature. Boxes were cleaned with 70% ethanol between mice and allowed to dry between rounds. The paradigm consisted of 1 habituation day (5 min exploration in neutral environment), 1 pre-training session (20 min with access to both chambers), 8 training days (biased subject assignment, alternating sessions of intraperitoneal injection of saline or ethanol immediately before the 5-min session) and 1 post-training test session (20 min with access to both chambers). The percentage of time spent in the conditioned chamber was measured by blinded investigators. Preference scores were calculated as the difference between the time spent in the conditioned chamber and the unconditioned chamber. A Shapiro–Wilk test was used to assess normal distribution and a Mann–Whitney test to determine learning.

Intracranial injection of viral vector
Adult male C57BL/6J mice of 10 weeks of age were anaesthetized with isoflurane gas (1.5–2% to maintain surgical plane) and placed in a sterile field within a stereotaxic device. Artificial teeth were applied to eyes to ensure sufficient lubrication. Mice received an injection of bupivacaine (2.5 mg kg −1) for local anaesthesia before the skin was disinfected with betadine solution and the skull exposed with a short incision using sterile surgical equipment. A small hole (about 0.5 mm) was drilled in the skull over the target area using a stereotax and a stereotactic drill. A micro-syringe filled with viral vector was inserted into the dHPC and slowly removed following injection (AP, −2.0 mm; DV, −1.4 mm; ML, ± 1.5 mm from bregma). The vector for ACSS2 knockdown was AAV2/9.U6.shACSS2.CMV.EGFP. All mice received a single dose of subcutaneous meloxicam (5 mg kg −1) as analgesia at induction and one dose per day for two days post-op eratively as needed.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.
Data availability

All RNA-seq and ChIP–seq data are available at the Gene Expression Omnibus (GEO) with accession number GSE122188. Raw mass spectrometry data are provided as Supplementary Table 2.

28. Linderstrom-Lang, K. Deuterium exchange between peptides and water. Chem. Soc. Spec. Publ. 2, 1–20 (1955).
29. Sidoli, S., Simithy, J., Karch, K. K., Kulej, K. & Garcia, B. A. Low resolution data-independent acquisition in an LTQ-Orbitrap allows for simplified and fully untargeted analysis of histone modifications. Anal. Chem. 87, 11448–11454 (2015).
30. Loos, M., Gerber, C., Corona, F., Hollender, J. & Singer, H. Accelerated isotope fine structure calculation using pruned transition trees. Anal. Chem. 87, 5738–5744 (2015).
31. Trefely, S., Ashwell, P. & Snyder, N. W. FluxFix: automatic isotopologue normalization for metabolic tracer analysis. BMC Bioinformatics 17, 485 (2016).
32. Fernandez, C. A., Rosiers, C., Des, Previs, S. F., David, F. & Brunengraber, H. Correction of 13C mass isotopomer distributions for natural stable isotope abundance. J. Mass Spectrom. 31, 255–262 (1996).
33. Nativio, R. et al. Dysregulation of the epigenetic landscape of normal aging in Alzheimer’s disease. Nat. Neurosci. 21, 497–505 (2018).
34. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359 (2012).
35. Chen, X. et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133, 1106–1117 (2008).
36. Cunningham, C. L., Gremel, C. M. & Groblewski, P. A. Drug-induced conditioned place preference and aversion in mice. Nat. Protocols 1, 1662–1670 (2006).

Acknowledgements

We thank the Metabolomics Core of the Diabetes Research Center (DRC) for providing the mass spectrometry quantification of metabolites; J. D. Rabinowitz (the Princeton Metabolomics Core director) and C. Jang for advice; and the Neurons R Us core of the Mahoney Institute for Neurological Sciences for preparations of primary hippocampal neurons. We especially acknowledge J. Whetstine for the suggestion to test whether the administration of alcohol to a pregnant female mouse leads to histone acetylation in the gestating fetal brain. G.E. was supported by The Brody Family Medical Trust Fund Fellowship in Incurable Diseases of The Philadelphia Foundation. This work was supported by NIH PO1AG031862 and NIH RO1AA027202.

Author contributions

P.M. and S.L.B. developed the primary hypothesis; P.M., G.E. and S.L.B. designed the project; P.M. and G.E. performed most of the experiments; P.M. planned the ethanol-d6-labelling mass spectrometry, which was performed together with R.N., G.E. and S.S. with support from B.A.G.; G.E. and S.S. performed the acetate-d3-labelling mass spectrometry; the RNA-seq was performed in vivo by P.M. and in vitro by G.E., with support from R.N.; R.N. conducted the ChIP–seq; G.D. analysed all ChIP–seq and RNA-seq datasets; G.E., S.I.L. and D.C.A. performed the behavioural characterization with support from E.A.H.; P.M. performed the labelling experiments for metabolomic analysis with support from E.J.N.; the fetal alcohol labelling was done by G.E., with support from S.L.R.; P.M., G.E. and S.L.B. wrote the manuscript. All authors reviewed the manuscript and discussed the work.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-019-1700-7.

Correspondence and requests for materials should be addressed to G.E. or S.L.B.

Reprints and permissions information is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Ethanol-derived acetyl groups are rapidly incorporated into histone acetylation in the brain. a, Mass spectrometry analysis of serum acetate shows the rapid increase in levels of acetate in mice that were injected with alcohol, at 30 min after injection (n = 3 for saline, n = 4 for acetate group). Data are mean ± s.e.m.; P = 0.0258 (two-tailed unpaired t-test).

b, Ethanol-d6 is readily metabolized and thus labels blood acetate pools. Acetate-d3 was detected by mass spectrometry (n = 4 per group). Data are mean ± s.e.m.; P = 0.0016 (two-tailed unpaired t-test).

c, Incorporation of the ethanol-d6 label into histone acetylation in the cortex shows a similar pattern to the hippocampus. The axis of the Arachne plot represents the percentage of the third isotope for the acetylated peptide, corresponding to the d3-labelled form; the natural relative abundance of that isotope is apparent in the ‘none’ and ‘saline 1 h’ treatment groups.

d, Histone acetylation is relatively independent of alcohol metabolism in skeletal muscle, a tissue in which the expression of ACSS2 is low.

e, f, Mass spectra (representative examples from three biological replicates) showing the relative abundance of deuterated histone H4-triacetyl peptide (amino acids 4–17) in the hippocampus of wild-type mice at baseline and 4 h after injection of ethanol-d6. Increases of the M+1 (blue lines), M+2 (green lines) and M+3 (red lines) species are shown in e and indicate a major increase of M+3. The contribution of singly (orange), doubly (grey) and triply (yellow) deuterated peptides to the isotopic distribution is shown in f. The relative abundance of the M+3 species is increased by about sixfold at 4 h after injection of ethanol-d6, and is overwhelmingly due to the triply deuterated peptides; by contrast, the contribution of singly and doubly deuterated peptides to the M+3 species is minimal. The experiment was performed with three biological replicates per group.

g, The relative abundance of the first four isotopes of each of the seven peptides in the untreated samples corresponds to the theoretical isotopic distribution of the peptides (calculated using enviPat30; samples not treated with ethanol-d6; n = 20). Data are mean ± s.d.

h, Natural abundance-corrected contribution of M+1, M+2 and M+3 species to the labelling of histone acetylation in the liver and hippocampus after intraperitoneal injection of ethanol-d6 (calculated using FluxFix31; n = 3 per group). Data are mean ± s.d.
Extended Data Fig. 2 | Dynamics of ethanol- and acetate-induced heavy-label incorporation. **a, b**, Relative abundance of deuterated histone acetylation in dHPC, vHPC, cortex, liver and muscle at 8 h (**a**) and 24 h (**b**) after intraperitoneal injection of ethanol-d₆. **c**, ¹³C-labelled ethanol, introduced by intraperitoneal injection, readily labels histone acetylation in the hippocampus (percentage increase over natural abundance of ¹³C acetyl groups in saline-injected mice; n = 1). **d**, In contrast to heavy ethanol-d₆, a non-labelled ethanol control does not increase the natural abundance of heavy histone acetylation in the hippocampus. **e**, Histone acetylation is relatively independent of alcohol metabolism in skeletal muscle. Relative abundance of deuterated histone acetylation in skeletal muscle tissue at 30 min and 4 h in wild-type mice, and 30 min in dHPC ACSS2-knockdown mice. **f, g**, Heavy acetate, introduced by intraperitoneal injection, readily labels histone acetylation in the dHPC (**f**) and the cortex (**g**) (n = 2 at 30 min; n = 3 per group at other time points). Data are mean ± s.e.m. **h**, Levels of acetate measured by mass spectrometry in hippocampal tissue after injections of acetate and ethanol (n = 3 per group). Data are mean ± s.e.m.; P = 0.0335 for acetate versus saline; P = 0.0285 for ethanol versus saline (two-tailed unpaired t test).
Extended Data Fig. 3 | Metabolite labelling in hippocampal tissue 30 min after intraperitoneal injection of ethanol-d₆. a–f. Mass spectrometry quantification of metabolite labelling in hippocampal tissue at 30 min after intraperitoneal injection of ethanol-d₆. The ethanol-d₆ label was incorporated into the acetate pool in the hippocampus (a). By contrast, ethanol-d₆ did not contribute to the glucose (b) or 3-hydroxybutyrate (d) pools in the hippocampus, and only minimally to the lactate pool (c). Labelling was observed in the hippocampal glutamine (e) and citrate or isocitrate (f) pools.
Extended Data Fig. 4 | Representative H3K9ac and H3K27ac dHPC ChIP-seq tracks in control and ethanol-treated wild-type and ACSS2-knockdown mice. a–c, ChIP-seq for H3K9ac and H3K27ac in untreated and ethanol-treated wild-type and ACSS2-knockdown mice. Genome-browser track views show the Cep152 locus (chr2: 125,603,000–125,626,000) (a), Uimc1 locus (chr5: 55,064,000–55,089,000) (b) and Nsmaf locus (chr4: 6,425,000–6,464,000) (c). The experiment was performed with three independent biological replicates per group.
Extended Data Fig. 5 | Epigenetic and transcriptional changes in the dHPC of control and ethanol-treated wild-type and ACSS2-knockdown mice. a–d, Decile plots of genes that are enriched in H3K9ac (a) and H3K27ac (b) show correlation with mRNA expression levels in hippocampus, in wild-type mice 1 h after injection with ethanol. By contrast, in ACSS2-knockdown mice, the correlation between histone H3K9 acetylation (c) and H3K27 acetylation (d) and alcohol-related mRNA expression is largely lost (n = 16,553 genes (population) arranged into ten equal-sized deciles by ChIP–seq enrichment of acetylation). Box plots as in Fig. 2. e, f, GO analysis on H3K9ac (e) and H3K27ac (f) peaks that are induced by ethanol in wild-type but not ACSS2-knockdown mice (n = 332 H3K9ac peaks and n = 480 H3K27ac peaks). GO enrichment analysis was performed using a modified Fisher's exact test (EASE) with the FDR corrected by the Yekutieli procedure; $-\log_{10}$ transformations of nominal P values are shown.
Extended Data Fig. 6 | Transcriptional changes in primary hippocampal neurons that were treated with supraphysiological levels of acetate.

a, Structure of ACSS2i (C_{20}H_{18}N_{4}O_{2}S_{2}).
b, RNA-seq showing differentially regulated genes in primary hippocampal neurons that were treated with 5 mM acetate (n = 4 replicates per group; volcano plot of likelihood ratio test (two-sided) in DESeq2; FDR controlled for multiple hypothesis testing).
c, d, GO analysis of genes that are significantly upregulated (c; n = 3,613 genes) and significantly downregulated (d; n = 3,987 genes) after treatment with 5 mM acetate. GO analysis was performed with GOrilla, using a minimal hypergeometric test.
e, RNA-seq in primary hippocampal neurons that were isolated from C57/Bl6 mouse embryos and treated with acetate (5 mM) in the presence or absence of ACSS2i. Of the 3,613 acetate-induced genes, 2,107 are not upregulated in the presence of ACSS2i (n = 3,613 induced genes (population) or 3,613 randomly sampled genes (population); P < 2.2 × 10^{-16} (two-sided Mann–Whitney rank-sum test)). Box plots as in Fig. 2.
f, Acetate-induced genes in primary hippocampal neurons are shown in blue (n = 3,613). Of these genes, 2,107 (non-overlapping with orange) were sensitive to ACSS2i, and 1,506 were also induced in the presence of ACSS2i (overlapping with orange). GO enrichment analysis was performed using a modified Fisher’s exact test (EASE) with the FDR corrected by the Yekutieli procedure; −log_{10} transformations of nominal P values are shown.
Extended Data Fig. 7 | Representative RNA-seq tracks in control and acetate-treated primary hippocampal neurons in the presence or absence of ACSS2i. a–d. Genome-browser track views showing examples of gene upregulation after treatment with acetate in hippocampal neurons, and diminished induction after treatment with ACSS2i (n = 4 per cohort). RNA-seq track views show the Slc17a7 locus (chr7: 45,162,500–45,179,000) (a), the Ccnj1 locus (chr11: 43,525,000–43,595,000) (b), the Cpne7 locus (chr8: 123,152,500–123,137,500) (c) and the Ndufv3 locus (chr17: 31,523,000–31,534,000) (d).
Extended Data Fig. 8 | Transcriptional changes that are induced by acetate in primary hippocampal neurons relate to in vivo ACSS2 peaks and in vivo changes in gene expression that are induced by ethanol. a, Cumulative number of ACSS2 peaks near the transcription start site of acetylated ACSS2i sensitive genes, indicating that the majority of ACSS2-binding events occur over or proximal to the gene promoter. b, GO analysis for the 830 overlapping genes between the in vivo RNA-seq and ex vivo hippocampal-neuron RNA-seq (n = 830 genes (population)). GO enrichment analysis was performed using a modified Fisher’s exact test (EASE) with the FDR corrected by the Yekutieli procedure.
Extended Data Fig. 9 | Behavioural importance of ACSS2 expression in the dHPC and heavy-label incorporation in the fetal brain. a, Representative image showing virus localization to the dHPC, and western blot (n = 4 mice) showing ACSS2 levels in the dHPC of wild-type and ACSS2-knockdown mice (for gel source data, see Supplementary Fig. 1). b, Quantification of the levels of ACSS2 protein in the dHPC and cortex of wild-type and dHPC ACSS2-knockdown mice (n = 4 mice). Data are mean ± s.e.m.; P = 0.0001 and q = 0.0001 for ACSS2-knockdown versus wild-type mice (dHPC); P = 0.2666 and q = 0.1347 for ACSS2-knockdown versus wild-type mice (cortex) (multiple t-test). c, ACSS2 is required for alcohol-induced associative learning. Mean time (seconds per minute) spent in unconditioned and ethanol-conditioned chambers following ethanol-induced CPP training in wild-type (n = 8) and dHPC ACSS2-knockdown mice (n = 10). Bar graphs represent mean ± s.e.m. and data points correspond to individual mice. d, Incorporation of ethanol-d6 into histone acetylation in the fetal brain. Data represent the second of two pools of embryos (n = 4 per pool) from maternal ethanol-d6 injection. The axes of the Arachne plots represent the percentage of the third isotope for the acetylated peptide, corresponding to the d3-labelled form.
Corresponding author(s): Shelley L Berger

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted
- Give $P$ values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's $d$, Pearson's $r$), indicating how they were calculated
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Software and code

Policy information about availability of computer code

Data collection

| Software used: STAR v2.5.2a |
|-----------------------------|
| HTSeq v0.6.1p1              |
| DESeq2 v1.22.2              |
| HOMER v4.6                  |
| DAVID 6.8                   |
| R 3.5.0 for statistical testing |
| DiffBind v1.8.5             |
| MACS2 2.1.0.2010616         |
| BEDtools v2.15.0            |
| bedGraphToBigWig v4         |

Data analysis

GEO SuperSeries GSE122188 [NCBI tracking system #19551484] for detailed information on software used (all publicly available)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All sequencing data is accessible on GEO; accession GSE122188

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](http://nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Sample sizes were selected in accordance with the literature and based on previous experience in our group. The Berger lab has experience assessing animal behavior with pharmacological or genetic manipulations and in our experience, robust effects are achieved when group sizes are at least >7-9 animals. Regarding reproducibility, all of our RNA-seq and ChIP-seq datasets were replicated 3-4 times for each condition. Two replicates of RNA-seq are recommended by ENCODE: [https://genome.ucsc.edu/ENCODE/protocols/dataStandards/ENCODE_RNAseq_Standards_V1.0.pdf](https://genome.ucsc.edu/ENCODE/protocols/dataStandards/ENCODE_RNAseq_Standards_V1.0.pdf).

Data exclusions

Pre-established exclusion criteria: mice with higher than 65% pre-training to either of the compartments in the CPP were excluded from training. All mice that were trained were included in the analysis (i.e. nothing was excluded after the experiment was run). Grubbs test was used to detect statistically significant outliers. One animal was excluded from the in vivo acetate deposition experiment due to failed i.p. injection (Extended Data Fig 2f/g, 30 min timepoint). No other data were excluded from the analyses.

Replication

RNAseq of primary hippocampal neurons was successfully performed on 4 biological replicates. In vivo alcohol and acetate injection in mice was successfully performed on 2-3 biological replicates per time point. Fetal heavy acetyl group incorporation in mice was successfully tested on 2 biological replicates (two independent pools of fetal brain tissue).

Randomization

All samples and animals were randomly allocated into experimental and control groups.

Blinding

Mass spectrometry analysis of heavy labeled samples was performed by blinded investigators. All animal testing was scored blindly.

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| **Involved in the study**       | **Involved in the study** |
| [x] Unique biological materials | [x] ChiP-seq |
| [x] Antibodies                  | [x] Flow cytometry |
| [x] Eukaryotic cell lines       | [x] MRI-based neuroimaging |
| [x] Palaeontology               |         |
| [x] Animals and other organisms |         |
| [x] Human research participants |         |
**Antibodies**

Antibodies used: H3K9ac antibody (Active Motif, cat # 39137; lot # 09811002); H3K27ac antibody (Abcam, cat # 4729; lot # GR323132-1)

**Validation**

Chip-validated antibodies. Relevant information and citations available on the manufacturers’ websites; both antibodies were tested by Chip analysis. Chromatin IP performed using the Chip-IT Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5x 106cell equivalents per Chip) using 10μl of Histone H5 acetyllys9 antibody or the equivalent amount of rabbit IgG as a negative control.

**Animals and other organisms**

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**

Male and female C57BL/6J mice of 10 weeks of age were used.

**Wild animals**

The study did not involve wild animals.

**Field-collected samples**

The study did not involve samples collected from the field.

**ChIP-seq**

**Data deposition**

- Confirm that both raw and final processed data have been deposited in a public database such as GEO.
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

**Data access links**

- GEO SuperSeries GSE122188 [NCBI tracking system #19551484] Reviewers can access all information using the following secure token: vnupekygbrqnnk

**Files in database submission**

- All raw sequencing data and processed data discussed in manuscript

**Genome browser session**

- Link to GEO accession

**Methodology**

**Replicates**

n = 4 per condition; 16 total

16 total

**Sequencing depth**

Libraries were multiplexed using NEBNext Multiplex Oligos for Illumina (dual index primers) and single-ended sequenced (75 bp) on the NextSeq 500 platform (Illumina) in accordance with the manufacturer’s protocol. ChIP-seq tags generated with the NextSeq 500 platform were demultiplexed with the bcl2fastq utility and aligned to the mouse reference genome (assembly GRCh38/mm10) using Bowtie v1.1.1 [3] allowing up to two mismatches per sequencing tag (parameters -m1 – best).

**Antibodies**

H3K9ac antibody (Active Motif, cat # 39137; lot # 09811002); H3K27ac antibody (Abcam, cat # 4729; lot # GR323132-1)

**Peak calling parameters**

ChIP-seq tags generated with the NextSeq 500 platform were demultiplexed with the bcl2fastq utility and aligned to the mouse reference genome (assembly GRCh38/mm10) using Bowtie v1.1.1 [3] allowing up to two mismatches per sequencing tag (parameters -m1 – best). Peaks were detected using MACS2 (tag size = 75 bp; FDR < 3x10-31 from pooled H3K9ac or H3K27ac tags of mice from the same condition along with treatment-matched input tags as control).

**Data quality**

The MUSC method was used to compare H3K9ac or H3K27ac enrichment in the four study conditions. Statistical significance of differential H3K9ac or H3K27ac enrichments was assessed by using DiffBind (Biocductor v3.7) in a 2-way comparison (wt-saline vs wt-EtOH or ACSS2KD-saline vs ACSS2KD-EtOH) across the individual replicate samples (FDR < 10%).

**Software**

UCSC Genome Browser track views were created for ChIP-seq data by first pooling replicates and generating coverage maps using BEDtools genomeCoverageBed -bg, then adjusting for library size using the RPM coefficient. Input signal was then subtracted from ChIP signal. Resulting tracks were converted from bedGraph to BigWig using the Genome Browser’s bedGraphToBigWig utility. RNA-seq tracks were created similarly, first splitting by tag orientation to the genomic reference strand and then creating coverage maps. Because of RPM adjustment might cause a large deformation in the transcriptome distribution, maps were adjusted for library size using the average signal coefficients size factor determined by DESeq2. Resulting tracks were converted to BigWig as ChIP-seq tracks were, and the + and - tags from a given sample were plotted as overlays in a track hub.