Deletion of Crtc1 leads to hippocampal neuroenergetic impairments associated with depressive-like behavior

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INTRODUCTION

Mood disorders (MD) are among the leading causes of disability worldwide [1, 2]. The difficulty in defining appropriate treatments relates to the fact that these complex, dynamic and multifactorial psychiatric diseases are poorly understood [3]. The way these diseases are approached complicates the identification of therapeutic targets: diagnosis is currently based on subjective signs and symptoms, rather than on objective biological or chemical measurements. In practice, there is an urgency to establish reliable biomarkers within a framework of personalized treatment approaches [4, 5]. Neuroimaging techniques, such as magnetic resonance imaging (MRI), spectroscopy (MRS) and positron emission tomography (PET) are promising tools to achieve this goal by providing brain-specific information [6]. Nevertheless, development and validation of neuroimaging markers for psychiatry require prior understanding of their underlying pathophysiological origin and the genetic and environmental factors linking these markers to the behavioral deficit [6]. Among many potential etiological factors that have been identified, metabolic syndrome (MeS), i.e., a combination of obesity, dyslipidemia, insulin resistance, and hypertension, has gained significant attention due to its high co-occurrence with MD [7–10]. However, the mechanisms and the causality relationship between peripheral metabolic alterations and dysfunction of the central mood regulation, and how this translates to in vivo brain measurements, remain to be fully elucidated.

The CREB-Regulated Transcription Coactivator 1 (CRTC1) gene has emerged as a promising target to study how features of MeS can lead to behavioral impairments. Several studies have identified a relationship between CRTC1 polymorphisms and psychiatric disorders, with focus on obesity parameters [11–14] and stress [15]. Through its enhancement of CREB transcriptional activity and because of its ability to sense both Ca^{2+} and cAMP second messengers in neurons, CRTC1 has been established as a key regulator of brain function and metabolism [16, 17]. CRTC1 is involved in synaptic plasticity and memory formation [18–20] and participates in the regulation of energy and mood balance [21, 22]. Importantly, CRTC1 has been implicated in rodent depressive-like behavior [23–26], which can be triggered by excessive CRTC1 phosphorylation and cytoplasmic sequestration as a response to chronic stress [27]. Thus, the Crtc1 knockout (Crtc1^{−/−}) mouse was shown to be a useful model to study the pathways and mechanisms linking metabolic diseases with depression [21, 22, 28, 29] and to understand associated resistance to classic antidepressants, in particular to fluoxetine [30, 31].
Here, using state-of-the-art preclinical neuroimaging technologies, we sought to identify fingerprints of brain metabolic disturbances in Crtc1−/− mice and to explore their mechanistic relationship with behavioral dysfunctions and MeS. By combining MRS, MRI and PET, we found that deletion of Crtc1 in mice uncovers hippocampal neuroenergetic markers that are associated with depressive-like behavior. By deciphering the pathophysiological mechanisms associated with these brain markers and behavior, we were able to select a targeted treatment, which reversed the pathological phenotype. Our results highlight new mechanisms linking Crtc1 and MeS with the development of depressive-like behavior, bringing to the forefront associated preclinical neuroimaging markers with clinical potential, and identification of a compatible mood-stabilizer with therapeutic capacity.

MATERIALS AND METHODS

Animals

Crtc1 knock-out (Crtc1−/−) mice and wild-type (WT, i.e., Crtc1+/+) littermates were bred and genotyped as previously described [30]. Mice were housed in standard Plexiglass filter-top cages in a normal 12 h day-light cycle environment at a temperature of 23 ± 1 °C and humidity of 40%. Animals had access to ad libitum standard rodent chow diet and water. Wearing of newborn mice was done at 21 days and followed by group-housing until being isolated at ~6 weeks to prevent injuries of cage mates. Weaning of newborn mice was done at 21 days and followed by group-housing until being isolated at ~6 weeks to prevent injuries of cage mates. Animals had ad libitum access to standard rodent chow diet and water. Light cycle environment at a temperature of 23 ± 1 °C and humidity of 40%.

Animals were socially isolated again after the first imaging time point and were then subjected to a 4 weeks stress and treatment (randomly assigned and blinded) protocol.

In vivo 1H-magnetic resonance spectroscopy (1H-MRS)

Localizad in vivo 1H-magnetic resonance spectroscopy (1H-MRS) was performed in the dorsal hippocampus (DH) and cingulate prefrontal cortex (PFC) of Crtc1−/− and WT mice. Animals were maintained under continuous isoﬂurane anesthesia (1.5% mixed with 1 air/oxygen mixture) and monitoring of physiology during the entire scan for physiological parameters. Breathing rate per minute was maintained between 70–100 rpm using a small animal monitor (SA Instruments Inc., New York, USA) and rectal temperature was kept at 36.5 ± 0.4 °C with a circulating heating water bath and assessed using a temperature rectal probe. Animals were anesthetized with 1.5% isoﬂurane and 1:1:1 mixture of CHCl 3:MeOH:H 2O for 30 min after what the aequous phase was collected and lyophilized. The resulting metabolites were resuspended in 600 μl deuterium oxide containing 0.1 μM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as internal reference. High-resolution NMR was performed using a DRX-600 spectrometer (Bruker BioSpin, Fällanden, Switzerland). Proton-NMR (1H-NMR) spectra were acquired with 400 scans using a pulse-acquired sequence (flip angle 30° and 5 s pulse delay). Phosphorus-NMR (31P-NMR) spectra were acquired on the same sample with 10,000 scans using a proton-decoupled pulse-acquired sequence (flip angle 90° and 5 s pulse delay). Spectra were analyzed and quantified using the MestReNova software (Mestrelab Research, Santiago de Compostela, Spain). Spectra were phased and baseline corrected manually. Afterwards, peaks were integrated and referenced to the DSS resonance and normalized to NAA. NAA concentration in DH was assumed to be 7 mM as measured in vivo. The following resonance (δ, in ppm) were considered (number of protons, spectral pattern): AX (sum of AMP, ADP and ATP) δ 6.13 (1H, d), creatine δ 3.026 (3H, s), phosphocreatine δ 3.028 (3H, s) and N-acetyl-aspartate δ 2.00 (1H, s). The following resonance were integrated in the 31P spectrum after setting the PCr resonance to 0 ppm: NAD + δ −8.31 (2P, q), NADH δ −8.15 (2P, m), UDPGlC δ −9.83 (2P, m), Pδ 3.8 (1P, m), GPC δ 3.07 (1P, s). The spectral pattern is described as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet. Due to overlap between resonances, the NAD+/NADH ratio was calculated metabolomics. The left part of the NAD + quadruplet (X = NAD + + NADH + − UDPGlC) was integrated as well as the right part of the quadruplet (Y = NAD +) and the −9.83 ppm UDPGlC resonance (Z = UDPGlc). Then, NADH was obtained by subtracting Y and Z from X, followed by a division by 2. As we did not see changes in GPC in the hippocampus between groups, we used this signal as an internal reference for 31P-NMR spectra quantification.

In vivo 18FDG positron emission tomography (18FDG-PET)

Dynamic non-invasive fluorodeoxyglucose positron emission tomography (18FDG-PET) was performed as described previously [38, 39]. Briefly, mice under 1–2% (vol/vol) isoflurane anesthesia in O2 were anaesthetized in the scanner after tail vein cannulation and remained monitored for temperature and breathing rate throughout the experiment. Imaging was performed after i.v. bolus injection of 18F-FDG (~50 μCi) through the tail vein catheter with the scanner under 25 s of a 50 min injection PET scan. After histogramming and image reconstruction with the Labpet software (Gamma Medica, Sherbrook, Canada), PMOD 2.95 software (PMOD Technologies, Zurich) was used for the determination of the heat-maps of standardized uptake value, defined as [mean ROI activity (kBq/cm 2)]/(injected dose [kBq]/body weight [g]). Regions of interest, i.e., hippocampus (2 × 5.5 mm 2), were manually drawn over one axial slice. Mathematical modeling of hippocampal glucose metabolism was performed as previously described [38, 39], using the radioactive decay-corrected activity density values in [kBq/cc]. Intergroup differences could not be attributed to differences in the amount of 18FDG entering the blood, body weight, nor to differences in the kinetics of the arterial input function.
Total RNA was extracted and purified from micropunches of DH using a RNeasy Plus Minikit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. NanoDrop Lite (Thermo Scientific, Wilmington, DE, USA) was used for the UV spectrophotometric quantification of RNA concentrations and purity assessment. cDNAs were obtained by reverse transcription of 1 µg of RNA samples in 50 µl reaction mixture containing 4 µl of 5X Reaction Buffer, 100 nM of Oligo(dT), 50 ng of Random hexamers (Applied Biosystems, Foster City, CA, USA). Real-time quantitative PCR was subsequently performed with cDNA concentrations of 0.16 ng/µl on a 96-well plate with SYBR Green PCR Master Mix (Applied Biosystems). The reaction started with a 2 min step at 50 °C and 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C, and 30 s elongation at 72 °C. The relative gene expression was determined using the comparative ΔΔCt method and normalized to β-actine and β2 microglobulin (B-2m) as housekeeping genes. The primers were used at a concentration of 250 nM and described in Supplementary Table 1.

Mitochondrial respirometry

Animals were sacrificed by rapid decapitation followed by DH dissection. The tissue was weighed, placed in a petri dish on ice with 2 ml of relaxing solution (2.8 mM Na, 7.2 mM K, 5.8 mM ATP, 6.6 mM MgCl2, 20 mM taure, 15 mM phosphocreatine, 20 mM imidazole, 0.5 mM dithiothreitol and 50 mM MES, pH = 7.1) until further preparation. Gentle homogenization was then performed in ice-cold respirometry medium (mRO5: 0.5 mM EGTA, 3 mM MgCl2, 60 mM potassium lactobionate, 20 mM taurine, 10 mM KH2PO4, 20 mM HEPES, 110 mM sucrose and 0.1% (w/v) BSA, pH = 7.1) with an Eppendorf pestle. Two mg of tissue were then used for high-resolution respirometry (Oroboros Oxygraph 2K. Oroborus Instruments, Innsbruck, Austria) to measure mitochondrial respiration rates at 37 °C. The experimental protocol consists in several experimental steps, which test the capacity of the different mitochondrial electron transport chain components by measuring the O2 flux in the sample. (1) The activity of complex I (CI) is measured by adding ADP (5 mM) to a mixture of malate (2 mM), pyruvate (10 mM) and glutamate (20 mM). Succinate (10 mM) is subsequently added to the medium to stimulate complex II and measure the capacity of both complexes (CI + CII). (3) Protonophore FCCP (carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone) is then used (successive titrations of 0.2 µM until reaching maximal respiration) to uncouple the respiration and provides information on the maximal capacity of the electron transfer system (ETS). (4) Rotenone (0.1 µM) was then used to inhibit complex I and quantify the contribution of complex II in the uncoupled state (ETS CII). (5) Antimycin (2 µM) is added to inhibit complex III and block the ETS in order to assess the residual oxygen consumption (ROX) provided by oxidative reactions unrelated to mitochondrial respiration. Oxygen fluxes were normalized by the wet weight of tissue sample and corrected for ROX.

Blood metabolite measurements

Blood sampling was performed after the last 1H-MRS scan of the longitudinal and treatment studies. Blood was collected from the trunk after head decapitation using collection tubes (Heparin/Li2 Microvette CB300 LH, Sarstedt). Samples were centrifugated at 1000 x g for 10 min at room temperature leading to ~100 µl of plasma, which was then frozen in liquid nitrogen and stored at −80 °C. Blood MeS markers were then quantified using an ELISA kit (insulin: EZBMI-13K, Millipore; glucose) and colorimetric assays (triglyceride: 10010303, Cayman; insulin: EZRMI-13K, Millipore; glucose) according to the manufacturer's instructions and with the following dilution factors: triglyceride: 1/2, insulin: 1/5, and glucose: 1/20. All mice and time-points. Finally, the behavioral composite z-score was calculated by averaging the two z-scores of TST and FST for each mouse time point.

Repeated open-space forced swim test (OSFST)

The repeated OSFST protocol was used as described previously [30, 31]. Animals were introduced into a cage (45 x 28 x 20 cm) filled up to ~13 cm with 34–35 °C tap water colored with milk. Mice were subjected to 4 consecutive days of swimming (day −9 to −6) for 15 min. Mice were then subjected to additional swim sessions for 3 weeks under treatment, according to the following interval: days −1, 3, 7, 10, 13, 17, 20. Water was replaced regularly between tests to ensure constant water temperature. Animals were videotaped from above and immobility time was recorded manually.

Ebselen treatment

Animals were treated with ebselen (Tokyo Chemical Industry, Tokyo, Japan) starting from day 0 until the end of the repeated OSFST protocol. Mice received oral administration (gavage) of ebselen (10 mg/kg) dissolved in 5% (w/v) carboxymethylcellulose (CMC; Sigma Aldrich) two times a day (mornings and evenings) for 21 consecutive days. The control group was administered a 5% CMC vehicle solution of the same volume. The dose was adjusted to any body weight gain.

Neuroimaging marker assessment

Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were used to establish cut-off from WT mice on the basis of their PFC alterations, which took into account the concentration of total choline (tCho) and tissue volume separately or as an average of individual z-scores. For this averaged score, the PFC individual z-scores were calculated using the whole sample average and standard deviation for each experiment combined (longitudinal and treatment). ROC curves and AUC were also established for the neuroenergetic profile (Lac and PCr) of DH for discriminating mice with “high” and “low” depressive-like behavior. In order to consider the different ways of assessing the behavior between the longitudinal (FST + TST) and treatment (OSFST) studies, a behavioral z-score was calculated using the sample average and standard deviation for each behavioral test separately. Subsequently, mice were separated into “high” or “low” depressive-like behavior, whether their score was higher (+z) or lower (−z) than the average, respectively. The ability of the DH neuroimaging markers to distinguish these two populations was tested using ROC curves for either Lac or PCr concentrations separately or as an average of individual z-scores.

In vivo indirect 13C magnetic resonance spectroscopy (1H-13C-MRS)

Non-invasive indirect carbon-13 magnetic resonance spectroscopy (1H-13C-MRS) was performed as previously described [39, 41]. The experimental set-up was comparable to that of 1H-MRS, with two main differences (1) animals underwent femoral vein cannulation for the
infusion of uniformly labeled $^{13}$C-glucose ($[U-^{13}\text{C}]_{6}\text{Glc}$) for a scan of ~230 min duration; and (2) the coil included a $^{13}$C channel. Breathing rate was maintained at ~80 rpm and rectal body temperature was kept at 36.2 ± 0.3 °C for both groups throughout the scan. Blood glycemia was measured before (GlcBlood(WT) = 7.7 ± 3.5 mM vs. GlcBlood(Crtc1−/−) = 7.3 ± 0.9 mM, n.s.) and after the infusion/scan (GlcBlood(WT) = 21 ± 4 mM vs. GlcBlood(Crtc1−/−) = 28 ± 13 mM, n.s.) using a Breeze-2 meter (Bayer AG, Leverkusen, Germany). At the end of the experiment, blood lactate levels (LacBlood(WT) = 7.7 ± 1.0 mM vs. LacBlood(Crtc1−/−) = 7.9 ± 0.9 mM; n.s.) were measured using two nearby GM7 analyzers (Analox Instruments Ltd, Stourbridge, UK). The VOI included the bilateral DH (2 × 5.5 × 1.5 mm³) and led to a typical water linewidth of 20 ± 1 Hz after field homogeneity adjustment. $^{1}$H-[${}^{13}$C]-MRS spectra were acquired using the full intensity SPECIAL-BISEP sequence (TE = 2.8 ms, TR = 4000 ms, averages = 8) as
Fig. 1 Deletion of Crtc1 is associated with a neuroimaging fingerprint of reduced hippocampal neuroenergetics. A T2-weighted image acquired for localized MRS (VOI including dorsal hippocampus: yellow rectangle), with a scale bar of 2 mm (left) and typical 1H-MRS spectrum acquired in the dorsal hippocampus (DH) of 6 weeks old mice at 14.1 Tesla (right). Metabolites in the spectrum include: 1. phosphocreatine (PCr), 2. creatine (Cr), 3. glucose (Glc), 4. lactate (L), 5. alanine (Ala), 6. glutamate (Glu), 7. glutamine (Gln), 8. γ-aminobutyric acid (GABA), 9. N-acetylaspartyl-glutamate (NAAG), 10. aspartate (Asp), 11. glycine (Gly), 12. myo-inositol (Ins), 13. phosphothanolamine (PE), 14. glycero phosphophorycholine (GPC), 15. phosphoinosithanol (PICh), 16. N-acetyl-aspartate (NAA), 17. glutathione (GSH), 18. ascorbate (Asc), 19. taurine (Tau) as well as macromolecules (mac). Spectrum is shown with 3Hz exponential apodization. B, C Quantification of DH neurochemical profile from 1H-MRS in wild-type (WT; n = 10) and Crtc1−/− (n = 6) mice, *p < 0.05, unpaired Student’s t test. Data are shown as mean ± s.e.m. D Typical high-resolution 1H-NMR spectrum of DH extracts acquired at 600 MHz with E quantification of AXP (sum of AMP, ADP and ATP), PCr and Cr in wild-type (n = 8) and Crtc1−/− (n = 8) mice, *p < 0.05, Mann–Whitney test. F Typical high-resolution 31P-NMR spectrum of DH extracts with G quantification of NADH/NAD+ ratio as well as PCr, α-ATP and inorganic phosphate (Pi) relative to the GPC resonance in wild-type (n = 8) and Crtc1−/− (n = 8) mice, *p < 0.05, Mann–Whitney test. All high-resolution data (E and G) are shown as mean ± s.d.

previously described [39, 42, 43]. The non-edited (proton, 1H) and inverted spectra (editing OFF and ON) were obtained using an interleaved acquisition and were subtracted in the post processing steps to obtain the edited spectra (protons bound to carbon 13, 13C[1H]). The non-edited spectra were quantified using a standard basis set for the neurochemical profile, while the edited spectra were fitted with a basis set that included simulated LacC3, LacC2, AlaC2 + C3, GluC4, GluC3, GluC2, GluC4, GluC3, GluC2, AspC3, AspC2, GABA4C, GABA3C, GABA2C and acquired spectra of glucose. In vivo 1H-13C-MRS enables to follow the fate of brain glucose and its incorporation in several brain metabolites infusion of [U-13C6]Glc.

The concentration curves of each metabolite were determined by multiplying the fractional enrichment (FE) with the total molecular concentration measured in the non-edited spectra. The total concentration was obtained in the same voxel from the 18FDG-PET experiments. Following fluxes were included in the 1-compartment model: tricarboxylic acid cycle (Vtca); a dilution flux from blood lactate (Vbal) and from blood acetate (Vbal); a trans mitochondrial flux (Vmt); and finally, a neurotransmission flux (Vnt). The estimated fluxes from the pseudo 3-compartment model included: a dilution flux from blood lactate and from blood acetate (Vbal); the pyruvate dehydrogenase activity of excitatory (Vped) and inhibitory (Vped) neurons; a neurotransmission flux for excitatory (Vnt) and inhibitory (Vnt) neurons; a neurotransmission flux for excitatory (Vnt) and inhibitory (Vnt) neurons; glutamate deacrylase activity (Vgda); and two exchanges between two Glu or two GABA pools (Vex) and Vex. Values of pyruvate dehydrogenase activity (Vped), glial tricarboxylic acid cycle (Vg) and glial trans mitochondrial flux (Vmt) were fixed to known values and glial Gln efflux (Vgln) was set equal to Vbal, as described in Cherix et al. [39]. The other parameters were calculated from the estimated fluxes through mass-balance equations, assuming metabolic steady-state (i.e., no net change in metabolites concentration over the experiment duration); the GABA TCA shunt (Vshunt = Vgad − Vnt); glutamine synthetase activity (Vgs = Vnt − Vmt + Vbal); total GABA TCA (Vgta = Vgta + Vshunt); total GABA (Vgta); and the oxidative cerebral metabolic rate of glucose (CMRGox = Vgta + Vgta + Vgta + Vgta)/2). The brain-to-blood lactate efflux was calculated (Vbal = Vbal − Lacbrain/Lacblood) using the lactate concentration measured in the hippocampus (Lacbrain = 2.5 ± 1.1 mM vs. Lacblood = 0.8 ± 0.5 mM; p < 0.05, Student’s t test), from the non-edited spectra quantification and the final blood lactate measurements (Lacblood). An allostatic load refers to an “excess” in physiological/cellular dynamic adapt to match energetic needs in response to external stimuli [44]. To assess the level of mitochondrial allostatic pressure, the relative “oxidative allostatic loads” for Crtc1−/− mice were calculated for excitatory and inhibitory neurons separately, considering neurotransmission activity relative to mitochondrial ATP production, using following equation: Relative excitatory load = (Vnt + Vped)/[ATPox]exc (cpx − °Vmt/VATPox)WT and relative inhibitory load = (Vnt + Vped)/[ATPox]inh (cpx − °Vmt/VATPox)WT, where Vnt, and Vped are the excitatory and inhibitory neurotransmission cycling activities respectively, and VATPox are the excitatory and inhibitory ATP production rates from mitochondria.

Statistics

Statistics were all performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA). All values are given as mean ± s.e.m. unless stated otherwise. p values of p < 0.05 were considered statistically significant. Metabolite data from high-resolution 1H- and 31P-NMR were analyzed with a non-parametric Mann–Whitney test. Longitudinal measurements (behavior and metabolites) were analyzed using two-way analysis of variance (ANOVA) with genotype and time as both factors. Gene expression and metabolic comparisons with two factors (genotype and treatment) were analyzed with two-way ANOVA and a Bonferroni post hoc test when appropriate. Data from the OSFFT behavioral measurements were analyzed with a two-way ANOVA with repeated measures followed by a Fisher LSD post hoc test [31]. Standard deviation of metabolic flux estimates was obtained from 300 Monte–Carlo simulations. Flux comparisons between Crtc1−/− and WT mice were performed with a permutation analysis with 2000 random permutations, followed by individual two-tailed Student’s t tests [45]. All the comparisons between Crtc1−/− and WT animals were performed with paired or unpaired Student’s t test.

RESULTS

Deletion of Crtc1 is associated with a neuroimaging fingerprint of reduced hippocampal neuroenergetics

We first determined whether deletion of Crtc1 in mice has measurable metabolic consequences in the brain using proton MRS (1H-MRS) and MRI. Animals were scanned at an early age (6 weeks) in a 14.1 Tesla scanner (Fig. 1A) to acquire MRI whole brain anatomical images and 1H-MRS spectra of DH and PFC. When comparing the neurochemical profiles of Crtc1−/− mice as compared to their WT (i.e., Crtc1+/+) littermates (Fig. 1B, C), hippocampal neuroenergetic alterations were noted, including a reduced ratio of phosphocreatine relative to creatine (PCr/Cr; p = 0.04) and decreased level of lactate (Lac; p = 0.02). Subsequently, to evaluate the PCr to Cr ratio measured in vivo, high-resolution 1H- and 31P-NMR of hippocampal metabolite extracts (Fig. 1D–G) was performed in another group of mice to further assess the drop in PCr (Fig. 1E; p = 0.04). In addition, an increase in inorganic phosphate was observed (Pi; p = 0.03), in line with higher PCr hydrolysis, while ATP levels and the NADH/NAD+ ratio were similar in both groups (Fig. 1G, n.s.). Interestingly, the neurochemical profile of PFC (Fig. S1A, B) did not indicate neuroenergetic alterations, but an increase in total choline (Cho; p = 0.0006), i.e., glycero phosphorycholine (GPC) and phosphocholine (PCO), in Crtc1−/− mice. This rise in phospholipid-related metabolites coincided with bigger prefrontal volume (Fig. S1C), as measured from MRI images, suggesting potential prefrontal inflammation. These distinct observations between PFC and DH could not be attributed to differences in Crtc1 brain regional expression, as total Crtc1 mRNA was comparable between both regions in the WT mice (Fig. S1D). Taken together, these results indicate that Crtc1 deletion affects hippocampal energy metabolism and prefrontal integrity, producing a measurable fingerprint using neuroimaging modalities.

Deletion of Crtc1 impacts hippocampal glycolytic metabolism with subsequent mitochondrial allostatic load

We next aimed to identifying the origin of hippocampal metabolic alterations by assessing glycolytic and mitochondrial energetic
function. Measuring brain glucose utilization with PET, upon infusion of $^{18}$F-fluorodeoxyglucose ($^{18}$FDG) radiotracer, revealed that Crtc1$^{-/-}$ mice have less glucose consumption in the hippocampus compared to controls (Fig. 2A–D). Accumulation curves of $^{18}$F in hippocampus, resulting from cellular incorporation of $^{18}$FDG into $^{18}$FDG-6P through the action of hexokinase, were clearly reduced in the Crtc1$^{-/-}$ mice (Fig. 2A, B), which was associated with a 20% lower cerebral metabolic rate of glucose obtained by mathematical modeling (CMR$_{Glc}$; Fig. 2C, D; $p = 0.0045$). Interestingly, the ability to produce energy through mitochondrial function did not appear to be affected per se, as we did not observe any significant alteration of electron transport
system expression (mtDNA- or nDNA-encoded; Fig. 2E) or respiration efficiency (Fig. 2F) in Crtc1−/− mice. Furthermore, no apparent difference in master regulators of mitochondrial biogenesis and function, i.e., PGC1α and β (peroxisome proliferator-activated receptor gamma coactivator 1α and beta), was observed (Fig. 2G), strengthening the idea that mitochondrial capacity is not directly affected by deletion of Crtc1. Nevertheless, the low PCr/P, ratio described earlier strongly suggests that mitochondria are under pressure to maintain homeostasis, as supported by creatine kinase (cytoplasmic, Ckmt1; and mitochondrial, Ckb) upregulation in young Crtc1−/− mice (Fig. 2G). In sum, these results suggest that the low hippocampal PCr and Lac content observed in young Crtc1−/− mice arises from impaired glycolytic metabolism, creating a pressure to maintain steady ATP levels (Fig. 2H), a situation described as an allostatic load.

Hippocampal energetic status reflects the depressive-like behavior of Crtc1−/− mice

To test the stability over time of these hippocampal energetic alterations and determine if they were associated with the depressive-like behavior of Crtc1−/− mice, we subjected WT and Crtc1−/− animals to social isolation from the age of 6 weeks and monitored their neurochemical profile and behavior longitudinally (Fig. 3A). Social isolation was used to produce a comparable social environment across both groups and reduce aggression-related effects within cages [30]. A higher level of depressive-like behavior was observed (Fig. 3B) for Crtc1−/− mice under basal conditions (6 weeks of age) as reflected in forced swim test (FST; p = 0.02) but not in tail suspension test (TST; n.s.). Surprisingly, 18 weeks of our social isolation protocol had an opposite effect on the behavior of the two groups (averaged z-scores, Interaction: F2,28 = 10.26, p = 0.0005; TST, Interaction: F2,28 = 5.16, p = 0.012; FST, Interaction: F2,28 = 3.87, p = 0.035; two-way ANOVA). Moreover, an inversion in the hippocampal energetic profile (Fig. 3C) coincided with this switch in behavior (Lac, Interaction: F2,28 = 7.32, p = 0.003; PCr, Interaction: F2,28 = 4.78, p = 0.017; PCr/ Cr, Interaction: F2,28 = 2.79, p = 0.08; two-way ANOVA). Interestingly, hippocampal glucose concentration rose only in Crtc1−/− mice upon social isolation (Time effect: F2,28 = 3.43, p = 0.050, two-way ANOVA; Crtc1−/− 6-weeks vs. 6-months, *p < 0.05, Bonferroni’s test). We then performed correlational analyses to further relate metabolite hippocampal markers with behavior (Fig. 3D) and found a significant negative correlation between the depressive-like behavior and Lac (Lac vs. averaged z-scores: R = −0.35, p = 0.01). To test whether these metabolic modifications were associated with a change in gene expression, we analyzed relative mRNA content in DH at the end of the protocol (Fig. 3E and Fig. S2A) and found a difference in Pgc1α (p = 0.04) and Glut4 (p = 0.01) between the two groups, while creatine kinases levels were no longer significantly different (n.s.). Notably, differences in depressive-like behavior between Crtc1−/− and WT mice were not related to locomotor activity at any age (Fig. S2B, n.s.) or PFC volume and tCho content, which both correlated with each other and remained increased in Crtc1−/− independently of the animal’s age (Figs. S2C and S3; tCho, Genotype effect: F1,148 = 12.89, p = 0.003; Volume, Genotype effect: F1,42 = 14.61, p = 0.004; two-way ANOVA; Correlation: R = 0.31, p = 0.03). Importantly, our social isolation protocol stimulated the development of a MeS-related phenotype in both groups as suggested by the rise in body weight (Fig. 3F), which developed faster over time for Crtc1−/− mice (Genotype effect: F1,14 = 5.84, p = 0.01; Interaction: F2,28 = 5.11, p = 0.03, two-way ANOVA), and the high level of blood MeS markers (insulin, glucose and triglycerides), which were not significantly different between the groups (Fig. 3G; n.s.). Overall, these results confirm that the hippocampal neuroenergetic status of Crtc1−/− mice reflects their depressive-like behavior and indicate an apparent dependence on the experienced environment.

Restoring hippocampal energy balance with energy-boosting ebselen mood-stabilizer rescues depressive-like behavior in Crtc1−/− mice

Social isolation appeared to be beneficial for Crtc1−/− mice, consistent with their known aggressive behavior and social impairments toward other individuals [30]. We thus hypothesized that a repeated open-space forced swim test (OSFST) protocol (Fig. 4A), which contains an environments- than social-stress component, would challenge neuroenergetics in both groups of mice. In parallel, we tested whether improving brain metabolism with an energy-stimulating compound would reverse the stressful effects of the OSFST. To maximize the translational relevance of our findings, we decided to treat animals by oral administration of ebselen, a neuroprotective and antioxidant compound [46] with comparable pharmacological properties as lithium (e.g., inhibitor of GSK3β and inositol monophosphatase) [47] and with a strong clinical potential [48, 49]. After 4-consecutive days of swimming sessions and establishment of a stable depressive-like behavior in all groups of mice, animals were treated with either ebselen or vehicle twice a day for 3 weeks. As expected, the depressive-like behavior, measured as immobility time in OSFST, was higher in Crtc1−/− mice over time (Fig. 4B; Genotype effect: F1,10 = 65.09, p < 0.0001, two-way ANOVA). Ebselen rescued the behavior of Crtc1−/− mice (Interaction: F1,10 = 41.84, p < 0.0001; Treatment effect: F1,10 = 5.45, p = 0.04, two-way ANOVA) and led to an improvement in hippocampal energy metabolism (Fig. 4C, D). More specifically,
Ebselen raised hippocampal PCR content (Fig. 4D; ΔPCr/Cr, Treatment effect: $F_{1,21} = 4.41, p = 0.04$; two-way ANOVA) compared to the untreated groups, but lowered lactate levels in Crtc1−/− mice at the end of the study protocol (Fig. 4C; Lac day 21, $p = 0.045$; unpaired t-test), in line with enhanced mitochondrial activity. Furthermore, the difference in energy metabolite content correlated with a difference in behavior (ΔPCr/Cr, R = −0.42, p = 0.02; ΔLac, R = 0.41, p = 0.01) suggesting that both events were linked (Fig. 4E).

Gene expression analysis (Fig. 4F and Fig. S4A) supports that ebselen stimulated DH mitochondrial function, as highlighted by a treatment
Fig. 3 Hippocampal neuroenergetic status reflects the depressive-like behavior of Crtc1−/− mice. A Experimental design, and timeline of the longitudinal protocol used involving social isolation. Wild-type (WT; n = 10) and Crtc1−/− (n = 6) mice underwent a set of behavioral tests including an open-field test (OF; day 1), a forced swim test (FST; day 2) and a tail-suspension test (TST; day 3) followed by a 1H-MRS scan on day 4. After this first set of experiments, animals were isolated at the age of 6 weeks and the whole procedure was repeated at 12 and 24 weeks of age. After the last 1H-MRS scan, animals were sacrificed, and hippocampal and plasma samples were collected for analysis.

B A switch in depressive-like behavior between Crtc1−/− and wild-type mice occurs after 18 weeks of social isolation as revealed by the inversion in immobility time in TST (right panel; Interaction: F2,28 = 5.16, p = 0.012), FST (center panel; Interaction: F2,28 = 3.87, p = 0.035) and averaged z-score of TST and FST (left panel; Interaction: F2,28 = 10.26, p = 0.0005). Two-way ANOVA, followed by Fisher LSD post hoc test; *p < 0.05, **p < 0.01. C Hippocampal neuroenergetic profile switches between Crtc1−/− and wild-type mice at the end of 18 weeks of isolation as revealed by the inversion of lactate concentration (left panel; Interaction: F2,28 = 7.32, p = 0.003), Pcr/Pcr ratio (center left panel; Interaction: F2,28 = 2.79, p = 0.08) and Pcr (center right panel; Interaction: F2,28 = 4.78, p = 0.017). Hippocampal glucose levels increased in the Crtc1−/− group only at the end of the 18 weeks of isolation (Time effect: F2,28 = 7.32, p = 0.005). Two-way ANOVA, followed by Bonferroni’s post hoc test; *p < 0.05, **p < 0.1. D Correlative analysis between depressive-like behavior and hippocampal energy metabolite content. A significant negative correlation between Lac and behavior was found when results from FST and TST were considered together (R = −0.351, p = 0.013). Color code represents Pearson’s correlation coefficient and the analysis included all longitudinal age time points. Pearson’s Rs are shown for each correlation with associated p value (uncorrected for multiple comparisons); *p < 0.05, **p < 0.001. E At the end of 18 weeks of isolation, hippocampal levels of Pgc1α mRNA were higher while Glut4 levels were lower in Crtc1−/− as compared to wild-type mice. Mitochondrial and cytoplasmic creatine kinases were not significantly different (n.s.) between the two groups. Unpaired Student’s t test, *p < 0.05. F Body weight of all animals increased significantly over time (Time effect: F2,28 = 123.2, p = 0.0001) but increased more in the Crtc1−/− group (Genotype effect: F1,14 = 5.84, p = 0.030; Interaction: F2,28 = 5.11, p = 0.013). Two-way ANOVA followed by Fisher LSD post hoc test *p < 0.05. G Plasma markers of metabolic syndrome (insulin, glucose and triglycerides) were high in both groups but not significantly different from each other (n.s.).

Effect observed in Pgc1α (F1,27 = 13.28, p = 0.009), Glut4 (F1,27 = 8.22, p = 0.001) and Ckm1 (F2,27 = 4.79, p = 0.04, two-way ANOVA) mRNA content. Interestingly, treatment did not affect Ckb expression, which was slightly increased in the Crtc1−/−/− group (Genotype, F1,17 = 3.79, p = 0.06, two-way ANOVA) echoing our results in basal condition. Note, Western blot analysis of creatin kinase revealed an overall effect on protein levels predominantly carried by the mitochondrial-type enzyme which was increased by ~60% in the Crtc1−/− group as compared to WT (Fig. S4B; U-MtCK: Genotype effect: F1,23 = 25.06, p < 0.0001). To test for direct involvement of CRTC1 in the transcriptional regulation of our genes of interest, we assessed CRTC1-gene interactions in the hippocampus of 8-week-old male mice using a small-scale chromatin immunoprecipitation sequencing (ChIP-seq) approach (ChIP-seq antibody validation service, Active Motif, Inc, Carlsbad, CA, USA). This experiment allowed us to validate two CRTC1 antibodies and to determine the presence or absence of CRTC1’s binding for up to 8 genes of interest. These preliminary results suggested that both Ckb and Ckm1 are under the direct regulatory control of CRTC1 (data not shown). Importantly, ebselen did not interfere with the increased body weight and high insulin and triglyceride levels in Crtc1−/− mice (Fig. 4G; Treatment effect, n.s.), confirming a brain-specific mechanism, neither did ebselen affect PFC volume and tCho concentration differences observed in Crtc1−/− mice (Fig. S4C–E). Finally, to assess the potential clinical relevance of the identified neuroimaging markers we determined their specificities and sensitivities using ROC curves (Fig. S4F, G). Prefrontal volume and tCho concentration were able to differentiate Crtc1−/− mice from their WT counterparts with an area under the curve (AUC) of up to 82% (95% CI 0.755–0.886), when combined into an averaged z-score. The ability of hippocampal neuroenergetic markers to differentiate mice with “high” levels of depressive-like behavior from those with “low” levels was more modest, with an AUC of up to 66% (95% CI 0.555–0.756), when combined into an averaged z-score. In summary, stimulating mitochondrial energy metabolism was able to rescue the depressive-like behavior induced by stress in Crtc1−/− mice, leading to neuroimaging-based modifications that followed the treatment response.

GABAergic dysfunction links impaired hippocampal glucose metabolism with depressive-like behavior in Crtc1−/− susceptible mice

Finally, to assess the relative brain cellular metabolic contributions, we acquired indirect 13C-carbon magnetic resonance spectroscopy (1H-13C-MRS; Fig. 5A) data to assess metabolic fluxes using mathematical modeling. Fractional isotopic 13C-enrichment (FE) of brain glucose and downstream metabolites revealed clear group differences in animals of 6 weeks in age (Fig. 5B) involving metabolites associated with glycolysis (U-Glc, LacC3), tricarboxylic acid (TCA) cycle (GluC4) and GABAergic neurons metabolism (GABAC2-4). When fitting the mathematical models to the 13C-labeling data (Fig. 5C and Fig. S5A), we found that reduced GABA labeling (Fig.6B) did not arise from an increase in GABA activity according to our model (GABAergic, NT/NT; 6-fold increase) but glutamatergic (VNT; 2-fold increase) neurotransmission. Importantly, the increase in GABA labeling (Fig. 6B) did not arise from an increase in GAD activity according to our model (VNT; 0.32 ± 0.06 for WT vs. 0.30 ± 0.08 μmol/g/min for Crtc1−/−, n.s.) but reflected a dilution originating from exchange between two GABA pools and possibly triggered by GABAergic neurotransmission recycling (Fig. 5C; VNT; 0.006 ± 0.0002 for WT vs. 0.007 ± 0.003 μmol/g/min for Crtc1−/−, p = 0.002), in line with a probable inhibitory neuron hyperactivity. Furthermore, despite the relatively higher drop of ATP production rate in excitatory (−35%) compared to inhibitory (−15%) neurons (Fig. 5D), the relative oxidative allostatic load calculated as the neurotransmitter relative to ATP production (see methods) indicated a ~2.7-fold higher load for inhibitory neurons (8.4x higher in Crtc1−/− mice) relative to excitatory neurons (3.1x higher in Crtc1−/− mice), suggesting that GABAergic inhibitory neurons might be more at risk.

To further determine if the GABAergic system is particularly impacted by hippocampal energetic impairments, we re-analyzed main GABAergic gene expression in our different experimental protocols. Interestingly, levels of Gad2 and parvalbumin (Pvalb) were strongly associated with the behavioral state of the animals (Fig. 5D–F). At the age of 6 weeks, Gad2 was lower in Crtc1−/− mice (p = 0.04) when depressive-like behavior was high (Fig. 3B), while it was increased after social isolation (p = 0.03; Fig. 5D) when the behavior was inverted as well (Fig. 3B). Importantly, ebselen restored the levels of both Gad2 (Interaction: F1,27 = 5.53, p = 0.03) and Pvalb (treatment effect: F1,24 = 4.28, p = 0.049) in Crtc1−/− mice after OSFST. Finally, Pvalb was the only gene that correlated directly
with the level of depressive-like behavior in both experiments (Social isolation: $R = -0.55$, $p = 0.03$; OSFST + treatment: $R = -0.69$, $p = 0.0001$). The above results suggest that the hippocampal GABAergic system might be mechanistically involved in the depressive-like behavior induced by neuroenergetic impairments.

**DISCUSSION**

Understanding how genetic and environmental factors interact in metabolic diseases and how they impact normal brain and behavior is central for better diagnosing and treating related MD. Because of its central role in regulating brain metabolism and its
strong association with features of MeS in psychiatric patients [11–14]. Crtc1 is a key candidate gene to understand how (neuro-) metabolic alterations can affect normal behavior. In this study, we have been able to identify reduced hippocampal energy metabolism in Crtc1-deficient mice that translated into measurable in vivo neuroimaging markers. We have demonstrated that these neurochemical impairments were associated with animal depressive-like behavior, which could be reversed with an energy-boosting treatment known for its mood-stabilizing properties. Finally, we provide evidence for a hyper-activation and allostatic load of the hippocampal GABAergic system that could mediate behavioral consequences of the observed neuroenergetic imbalance.

Even though Crtc1 is predominantly expressed in the brain [30, 50] directing this gene in mice induces a somatic metabolic deregulation [22], such as insulin resistance and obesity, together with a depressive-like phenotype [30, 31]. As male Crtc1+/− mice show a stronger depressive-like phenotype with a more severe comorbid obesity than females [21, 23, 29], we decided to capitalize on the former to focus on mechanistic aspects rather than sex differences in our neuroimaging study. While the association of MeS and behavioral alterations is likely to be complex and multifactorial, we report a clear link between brain glucose uptake and depressive-like behavior. Specifically, low glycolytic activity in Crtc1+/− mice was associated with reduced levels of lactate and increase in high-energy phosphate hydrolysis (i.e., high level of Pi and low levels of PCr) in hippocampus that correlated well with animal behavior (Fig. 1). This imbalance in PCr/Cr level appeared to be driven by changes in creatine kinases expression, which might be directly regulated by CRTC1, as shown by our ChIP-seq preliminary data. Importantly, our results indicate reduced hippocampal glucose uptake capacity rather than lower demand, as the neuronal activity relative to energy production (V_{NEF}/V_{GCA}) was found to be ~3-fold higher for the Crtc1+/− mice (Fig. 5A–C and Fig. S5), pointing toward a difficulty in matching energy production with neuronal needs, or what is defined as an allostatic load [44]. This fits well with the idea that CRTC1 is required for adapting energy homeostasis according to neuronal requirements [22, 51] and is in line with several studies demonstrating the central role of brain energy metabolism in the resilience mechanisms against depressive-like behavior [52–56]. Of note, our results indicate that both glycolytic and mitochondrial pathways are fundamental for brain metabolic resilience and behavior rather than one route preferentially, as CMR_{Glc} deficiency in Crtc1+/− mice could be compensated by an increase in oxidative metabolism.

Considering that glucose entry in the brain is regulated by factors such as the insulin or IGF-1 receptors, known to influence mouse depressive-like behavior [57], we hypothesize that reduced hippocampal glucose uptake arises from the known insulin resistance phenotype of Crtc1+/− mice [22]. While future research will determine the exact molecular mechanisms relating Crtc1 with brain energy capacity, our experimental data point toward MeS as a player in this process. In fact, treatment with ebselen, known to inhibit GSK3β, improved DH energetic status and behavior through enhanced hippocampal Pgc1α and Glut4 expression, with only little effect on peripheral energy markers (Fig. 4G). PGC1α, as a master mitochondrial biogenesis regulator, can be inhibited through phosphorylation by GSK3β [58], possibly impacting Glut4 expression over the MEF2C transcription factor [59]. PGC1α has been linked with depression [60] and bipolar disorders [61], and its target, PPARγ, provides a plausible link between MeS and behavior. For instance, PPARγ agonists, which are well known insulin sensitizing agents [9], show anti-depressant properties in animal models [62] and patients [63, 64] leading to improved glucose metabolism [65]. Although studies focusing on muscle cells showed that Crtc2, a peripheral homolog of Crtc1, can induce Pgc1α expression [66], we did not observe reduced Pgc1α levels as a result of Crtc1 deletion (Figs. 2F, 3E and 4F). Nevertheless, enhancing Pgc1α expression restored energy metabolism and behavior in Crtc1+/− mice (Fig. 4F), suggesting that CRTC1 deficiency can be compensated through different, though converging, pathways. Of note, elevated mRNA levels of Pgc1α, but not Glut4, were associated with improved neuroenergetic profile at the end of the longitudinal study (Fig. 3E), suggesting
that simultaneous expression might not be required to improve behavior. Finally, despite the likely complex and multifactorial interplay of neuroenergetic genes, our results highlight a possible transcriptional control of CRTC1 on both Ckb and Ckmt1, confirming its direct mechanistic involvement in hippocampal metabolic regulation.

How then did the enhanced hippocampal energetic capacity, illustrated by higher Pgc1α and Glut4 expression (Fig. 4F), not affect the behavior of WT mice, as would be expected from this model? It is plausible that efficient energy metabolism is necessary for resilience to depressive-like behavior but is not sufficient to modulate it. Energy metabolism, either mitochondrial or glycolytic, has been widely implicated in the pathophysiological mechanisms leading to depressive-like behavior in preclinical models [52, 54–56, 67] and in clinical studies [68–71]. Nevertheless, it remains unclear how altered brain energy production rates could
translate into behavioral dysfunction. While several processes have been brought forward, such as metabolic, neuroendocrine-, inflammatory-, transcriptional-, or other responses [72–74], our results highlight the hippocampal GABAergic neurotransmitter system as a new key player in the process linking cellular allostatic load with affected neuronal output. In fact, our metabolic flux and GABAergic gene expression analyses indicate that the inhibitory system is particularly affected by low energy status and could relate to depressive-like behavior more tightly than the level of metabolism-enhancing genes or high-energy phosphates. We have previously reported that inhibitory neurotransmission in the hippocampus has high mitochondrial oxidative dependence compared to excitatory neurotransmission in mice [39]. Accordingly, here we found that low energy production capacity in Crtc1−/− mice was associated with a ~6-fold increase in hippocampal GABAergic neurotransmission cycling (Fig. 5A–C), leading to an overall higher (~2.6-fold) oxidative allostatic load in inhibitory compared to excitatory neurons. Others have shown that GABA neuronal metabolism is highly controlled by the cellular energetic status, through the action of both GAD isozymes (GAD65 and GAD67), switching from an Apo (inactive) to a Holos (active) conformation in response to low energy metabolism concentration, i.e., increased Pi or reduced PCr or ATP [75–77]. This feature would provide a protective network-inhibition mechanism when energy demands exceed metabolic capacities. Furthermore, our present work also shows that GABAergic markers (Gad1, Gad2 and Pvalb) were highly correlated with the animals’ behavior (Fig. 5D–F). Considering that fast-spiking parvalbumin-positive interneurons, particularly activated during gamma-oscillations in the hippocampus, are known to be very energy consuming and mitochondria-rich [78], improving energy metabolism might confer significant resilience to this cell population in particular. Of note, Uchida et al. reported that disruption of Gad1 function can lead to the loss of parvalbumin neurons in the hippocampus as a result of stress exposure [79]. Interestingly, several studies have also reported lower post-mortem levels of Gad2 expression in PFC of bipolar and schizophrenic patients [80–84]. Importantly, and of potential therapeutic relevance, ebselen was able to rescue the behavior in Crtc1−/− mice, by restoring hippocampal energy metabolites and levels of Gad2 and Pvalb expression (Fig. 4). This resonates with previous reports of increased GABA metabolism enzymes expression in hippocampus after ebselen treatment [85]. Given its synaptic location and dynamic regulation, Gad2, encoding the GAD65 isozyme, is likely to play a critical role in linking metabolic with electrophysiological activity (Fig. 5D, E). While it remains to be tested whether relative GAD conformation was altered and whether the rise in neurotransmitter cycling affected electrical activity in Crtc1−/− mice, we speculate that the low Pi and PCr observed must create a shift from Apo- to Holos-GAD, which would drive a compensatory drop in mRNA level, as observed here, allowing this enzyme to maintain a stable rate of GABA synthesis (Fig. 5G). This process would in turn favor the recycling of GABA for and from inhibitory neurotransmission rather than synthesis from glutamate, providing a mechanism to avoid excessive energy expenditure coming from extra metabolic steps, particularly when energy resources are low (Fig. 6). Although it is clear that Parvalbumin neurons are involved in this process, future mechanistic studies should address whether changes in Pvalb expression reflect differences in signaling within these neurons, such as involving BDNF-TrkB [86], or actual loss of neurons. Importantly, parvalbumin interneurons have been reported to be devoid of Crtc1 [19], suggesting that their dysfunction might not be directly related to Crtc1 deletion. Moreover, it is important to note that glutamate fluxes were assumed to be similar between our groups, due to the technical limitations of [U-13C]-glucose in distinguishing astrocytic contribution. Although it is known that CRTC1 is present in astrocytes [87], the consequences of its deletion in these cells remain to be identified. Nevertheless, with our current model, an increase (although not significant) in Glx exchange flux between two astroglial pools was observed, providing new avenues for understanding the role of glial cells. For instance, future experiment using 13C substrate will provide further insight into the implication of astrocytes in Crtc1-related hippocampal energy imbalance [88].

With the help of neuroimaging technologies such as fMRI, MRI and PET we have identified potential clinically relevant biological markers with their associated environmental dependences, opening potential therapeutic strategies. Using high-field 1H-MRS we observed a drop in energy metabolites PCr and lactate in the hippocampus (Fig. 1) that were associated with depressive-like behavior (Fig. 3), suggesting their potential use as psychopathological “state” markers. While both metabolites were found to be


demonstrated by the significant reduction of neuronal metabolic rate of glucose (CMRGlu) 

Observed a drop in energy metabolites PCr and lactate in the 

expressed in the hippocampus of a 6 weeks old WT mouse (right) as shown with the selected VOI (yellow box) on the associated MRI image (lower left). The non-edited spectrum (top) shows the total metabolic profile, while the edited spectrum (bottom) identifies the fraction of metabolites that have incorporated 13C-labeling. Scale bar = 2 mm. B Fractional isotopic 13C-enrichment (FE) of glucose and key metabolites in the hippocampus during 1H-[13C]-MRS experiment. Fitting of the data with a pseudo 3-compartment model of brain glucose metabolism is shown with a straight line for wild-type (WT; in blue) and Crtc1−/− (in red) mice. 6 weeks old wild-type (n = 8) and Crtc1−/− (n = 8). Data presented as mean ± s.d. C Schematic representation of hippocampal glucose utilization differences between wild-type and Crtc1−/− mice after metabolic flux analysis using a pseudo 3-compartment model. Metabolic fluxes that were different in Crtc1−/− animals (compared to their wild-type littermates) are shown in red, while those found lower are shown in blue and those found without any difference or fixed during the modeling remain in black. Cerebral metabolic rate of glucose (CMRGlu); brain lactate influx (Vlact in) and outflux (Vlact out) from blood; pyruvate flux (Vpyr); excitatory neurotransmitter TCA cycle (VεNTCA); inhibitory neurotransmitter pyruvate dehydrogenase activity (VεPDH); GABA shunt (Vshunt); inhibitory neuron TCA cycle (VεTCA = VεNTCA + VεPDH); glial pyruvate carboxylase (VPC); excitatory neuron (Vε); inhibitory neuron (Vi) and glial (Vg) transmitochondrial fluxes; excitatory neurotransmission flux (Vε); inhibitory neurotransmission flux (Vi); glutamate decarboxylase activity (VGDH); Gln exchange flux (VεGln); GABAergic exchange flux (VεGAD); glutamine synthetase activity (VGSN) and Gln efflux (VεGln). Relative flux increase/decrease is indicated for Crtc1−/− mice compared to WT littermates, as calculated from fluxes in mmol/g/min from Fig. 5C; and an asterisk (*) indicates a statistically significant difference between the two groups. D GABAergic gene expression (Gad1, Gad2 and parvalbumin (Pvalb)) in the hippocampus under basal conditions (6 weeks age; left) or after social isolation (24 weeks age; right). Unpaired Student’s t test, *p < 0.05; basal, wild-type (n = 6) and longitudinal, wild-type (n = 10) and Crtc1−/− (n = 6). E Hippocampal gene expression of Gad1, Gad2 and Pvalb after the OSFST protocol (wild-type(VEH), n = 9; wild-type(EBS), n = 8; Crtc1−/− (VEH), n = 9; Crtc1−/− (EBS), n = 6). Gad1 was significantly reduced in the Crtc1−/− group (Genotype effect: F1,28 = 4.39, p = 0.045, two-way ANOVA), while ebselen treatment increased the levels of Gad2 (Interaction: F1,27 = 5.53, p = 0.026, two-way ANOVA; *p < 0.05, Bonferroni’s post hoc test) and parvalbumin (Treatment effect: F1,24 = 4.28, p = 0.049, two-way ANOVA). F Correlation between depressive-like behavior and level of Pvalb expression in the hippocampus after social isolation (left; 24 weeks of age; R = −0.55, p = 0.03) and OSFST protocols (right; 10 weeks of age; R = −0.69, p = 0.0011). The dotted lines represent the 95% confidence interval of the linear regression line. G Scheme of potential relation between GAD expression level, energy metabolite binding and enzyme activity.

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lower in Crtc1−/− mice under basal conditions (i.e., 6 weeks of age; Figs. 1, 3C and 4C) and associated with reduced glucose uptake measured with PET (Fig. 2A–D), the addition of an external stressor (social isolation or OSFST) was able to modulate both the behavior and these in vivo markers (Figs. 3 and 4). Stress, by challenging brain energetics, was shown to impact brain PCr content and behavior in chronic social defeat or chronic restraint protocols in mice [53, 56]. Social isolation is known to affect the behavior in other rodents as well [89–91] and induces several biological dysfunctions such as oxidative damage [92], a loss of hippocampal parvalbumin neurons [93] or drop in PCr content [94, 95]. Accordingly, PCr and lactate levels appeared to relate tightly to the level of stress experienced and stimulating mitochondrial metabolism with ebselen was able to restore normal PCr levels from WT mice (AUC: 82%), suggesting a potential use for our observations in the DH and PFC could serve, respectively, as potential “diagnostic” and “predictive” clinical biomarkers for MD. Finally, by identifying how in vivo brain markers associated with Crtc1 respond to the environment, we provide a better characterization and understanding of the factors that influence the path from gene to depressive-like behavior, providing a hopeful step forward toward a precision medicine-based approach in the field of psychiatry.

Fig. 6 Scheme of hippocampal GABAergic hyperactivity resulting from low energetic status linking Crtc1 deletion to depressive-like behavior. Reduced hippocampal glucose metabolism capacity relative to neuronal neurotransmitter cycling-demands leads to low energetic status (high inorganic phosphate (Pi) and low phosphocreatine (PCr) levels) in Crtc1 deficient mice. This results in excessive GABAergic neurotransmitter cycling and depressive-like behavior.

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AUTHOR CONTRIBUTIONS

AC and JRC designed the study. AC, CPY, BL, OZ and JG acquired and analyzed the data. AC, CPY, CS, RG and JRC interpreted the data. AC drafted the manuscript. All the authors assisted in revising the manuscript and approved the final version.

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The authors declare no competing interests.

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