



c-Jun N-terminal Kinase 1 ablation protects against metabolic-induced hippocampal cognitive impairments

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Abstract

The development of metabolic alterations like insulin resistance has been associated with dysfunctions in mitochondrial oxidative capacity, induction of neuroinflammatory responses, and the appearance of cognitive impairments in the brain. The c-Jun N-terminal Kinase 1 (JNK1) is a potential key modulator of these mechanisms. The current study identifies a protective effect of whole-body JNK1 knockout in the presence of a high-fat diet (HFD). Specifically, the data suggest that mice missing JNK1 show increased insulin sensitivity and mitochondrial activity, as well as reduced body weight, and astrocyte and microglial reactivity. Finally, these animals are also protected against HFD-induced cognitive impairments as assessed through novel object recognition test, the observation of dendritic spines, and the levels of BDNF or other proteins like spinophilin and ARC. Thus, modulation of JNK1 activity seems like a promising approach for the design of therapies aimed at treating metabolic-induced cognitive impairments.

Key messages

- JNK1 is a link between obesity/type 2 diabetes and cognitive loss
- Inhibition of JNK1 is neuroprotective
- JNK1 constitutes a therapeutic strategy for cognitive loss.

Keywords c-Jun N-terminal Kinase 1 · Cognitive impairments · High-fat diet

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Introduction

Mild cognitive impairment is a human syndrome characterized by the appearance of small cognitive complaints that do not affect the performance of simple tasks [1]. It usually occurs in patients over 65 years old, can aggravate over time, and eventually leads to the appearance of dementia after 1 year [2]. Longitudinal studies in humans have observed how this progression is linked to dysregulations in the metabolism of glucose, a characteristic of diabetic alterations [2].

The nervous system depends on the metabolism of glucose to maintain its physiological activity [3]. In preclinical studies, alterations in the oxidation of this carbohydrate have been linked with a decrease in lifespan and the appearance of slow-building affectations that cause neurodegeneration [4]. It has been described that the insulin receptor (IR) and its signalling pathway play a role in the modulation of these mechanisms. Also, IR has been studied for its importance in the development of diabetic complications and proper functionality of cognition-related areas like the hippocampus and the prefrontal cortex [5]. On this note, Grillo and colleagues showed how silencing of the gene for IR in the hippocampus caused major spatial learning impairments when using antisense sequences in lentivirus [6]. These results correlated with previously reported studies in which sporadic forms of Alzheimer's disease have been associated with the desensitization of the IR [7, 8], a paradigm that has later been labelled as type 3 diabetes (T3D) [9, 10]. Thus, investigating mechanisms for the modulation of this pathway may prove relevant to identify new approaches to treat these afflictions.

The c-Jun N-terminal Kinases (JNKs) are very active stress-response elements that participate in the control of many cellular mechanisms [11]. Short-term activation is believed to be necessary for survival and the maintenance of physiological functions, while long-term activation has been described to cause the appearance of cellular stress and the induction of pathophysiological mechanisms. For example, it has been reported that there is high JNK activity in the hypothalamus during obesity [12, 13]. In 2010, Sabio and colleagues reported that isoform 1 (JNK1) is a highly active JNK isoform [14]. High JNK1 activity has been linked with the appearance of reactive stress responses, conditions like obesity, and pathologies like diabetes or anxiety and neurodegenerative disorders [15, 16]. On a molecular level, JNK1 is activated by cytokines, mitochondrial and endoplasmic reticulum stress, and hyperlipidaemia among many other stimuli, all of which are hallmarks of metabolic pathologies [4]. Furthermore, JNK1 regulates inhibitory serine phosphorylation of the IR substrate (IRS) proteins, which impairs insulin signalling. Yet, it has been observed that blocking of the Ser307 residue, a point of JNK1 activity, does not avoid insulin resistance but rather causes for a further increase, indicating the existence of multiple parallel and redundant

mechanisms through which JNK1 promotes metabolic alterations when it is activated [15, 17].

In order to study these mechanisms, whole-body knockout animals for JNK1 (*Jnk1*^{-/-}) have been previously used by several authors. It has been reported that JNK1 is necessary for the accumulation of visceral fat, and thus, its absence is protective against obesity, enhances sensitivity to insulin, and induces antiinflammatory effects in models of obesity induced with a high-fat diet (HFD) [16, 18]. Studies revealed that these animals showed metabolic protection against the effects of HFD for over 40 weeks and maintained a high level of tolerance and protection against oxidative damage in the adipose and hepatic tissues [17]. Controversially, Becattini and co-workers reported that this genetic modification, while beneficial for the control of peripheral metabolic alterations, caused for mild oxidative damage in the skin of mice at the age of 11 and 20 months when exposed to a HFD, yet effects were lower than in previously studied models like *Drosophila* or *Caenorhabditis elegans* [17]. Finally, researchers like Mohammad H and colleagues described how *Jnk1*^{-/-} animals showed lower anxiety levels and increased neurogenesis [15]. Similar results on the differences of neurogenic activity of the JNK1 transgenic animals have been reported by our research group [19]. Complementarily, tissue-specific knockouts of the JNK1 in the adipose tissue, muscle, and liver have also been described. In all cases, animals showed amelioration of metabolic alterations, but effects were not as significant as those produced by the whole-body knockout [20–22]. Exceptionally, conditional neuron JNK1 knockout mice showed dramatic sensitivity to insulin in the brain and the periphery and reduced inflammatory responses and complete protection against HFD in the hypothalamus [23]. Authors described very high energy expenditure rates, caused by the increased production of triiodothyronine (T3) and thyroid-stimulating hormone (TSH) hormones [23, 24].

Consequently, the present research is intended to validate the hypothesis of a molecular regulatory factor linking metabolic dysregulations and cognitive loss, to demonstrate the role of JNK1 isoform in these mechanisms, and suggest a putative target for future pharmacological strategies.

Research design and methods

Animals and diet

Male C57BL/6J wild-type (WT) and *Mpak8* montransgenic knockout animals were used for this study. The *Mapk8* gene codifies for the protein JNK1 (*Jnk1*^{-/-}). Transgenic animals were obtained and characterized following the method described by Dong and colleagues [25]. All animals used in this study were obtained from established breeding couples in the

animal facility (Pharmacy and Food Sciences Faculty, University of Barcelona; approval number C-0032).

The animals were fed with either control (CT; 12% of Kcal derived of fat content; Envigo; product 2016) or palmitic acid-enriched diets (high-fat diets (HFD); 45% of Kcal derived of fat content; Research Diets, Inc.; product D12451) from their weaning until they were euthanized. Thus, four experimental groups were established: WT CT, WT HFD, *Jnk1*^{-/-} CT, and *Jnk1*^{-/-} HFD. Animals were randomly assigned into each experimental group in a non-blinded manner, and 12–15 animals were used per group. They were grown until 9 months of age and underwent monthly weight controls. Environmental conditions of temperature and humidity were kept stable. Also, animals were kept under a 12-h light/dark cycle and had food and water available at all times (Pharmacy and Food Sciences Faculty, University of Barcelona). During all procedures, the European Committee bioethics directives were followed (European Communities Council Directive 2010/63/EU) and all protocols were previously approved by the ethics committee from the University of Barcelona. In all cases, it was made sure that animal numbers, stress, and pain were kept under a necessary minimum.

Glucose tolerance test–insulin tolerance test

Tests were conducted as previously described [26]. In short, animals were fasted 6 h previous to the tests and posteriorly injected with either glucose (1 g/kg) or insulin (0.75 UI/kg) in the intraperitoneal cavity. Peripheral glucose concentrations were calculated using a glucometer (Accu-Chek, Roche) at different time points right before (basal) and after the administration: glucose tolerance test (GTT) (5, 15, 30, 60, 120, and 180 min) and insulin tolerance test (ITT) (15, 30, 45, 60, and 90 min). Animals were monitored throughout the test, and if any of the subjects dropped below 20 mg/dl in the ITT, they were administered a dose of glucose (1 g/kg). Blood glucose levels and behaviour were checked regularly until they were stable.

Novel object recognition test

Experimental procedure was adapted from a publication by Bevins and Besheer [27]. The week previous to the test, animals were handled for a few minutes every day in order to reduce manipulation stress. To reduce environmental cues, tests were conducted in an open-field box (50 × 50 × 20 cm) surrounded by black curtains. Initial testing consisted of 3 days in which the animals were introduced to the open-field box for 10 min and were allowed to explore it freely (habituation period). Motor activity and stress for each animal were evaluated by the quantification of the speed, total distance, and time spent in the inner quadrant. On the following day, two identical objects were introduced into the open field and the exploration time for each object was quantified. Animals that showed

significant preference for one of the objects over the other were excluded. The next day, one of the objects was substituted by a new one and exploration time was quantified again.

During the experimental procedure, all spaces and objects were properly cleaned previous to the introduction of the animals in order to eliminate odour cues. Objects were randomized by blindly choosing from a box in each session so as to reduce any possible preference effects caused by colour or shape. All recordings and data were obtained using the program Smart 3.0 (Panlab). Motor activity and stress data was presented as a curve in which the mean and standard deviation were presented. Area under the curve was extrapolated for posterior statistical analysis. Discrimination ratio (DI) was calculated using the following formula: $DI = (\text{time spent exploring the new object} - \text{time spent exploring the known object}) / \text{total exploration time}$.

Western blot

Protein detection was performed from protein extracts of hippocampal tissue of mice euthanized by neck dislocation. Protein extraction and posterior western blot assays were performed as previously described [26]. References for the antibodies used for these assays have been described in Supplementary Material 1. Detections were performed through chemoluminescence using Pierce[®] ECL Western Blotting Substrate (#32106, Thermo Scientific, USA), a Bio-Rad Universal Hood II Molecular Imager, and the Image Lab v5.2.1 software (Bio-Rad Laboratories). Measurements were expressed in arbitrary units and all results were normalized with the corresponding loading control (glyceraldehyde-3-phosphate dehydrogenase (GAPDH)).

Immunofluorescence

Prior to perfusion with 4% paraformaldehyde, animals were anaesthetized through an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Posterior brain fixation, sectioning, and labelling through immunofluorescence have been previously described [26].

Antibodies used for IF have been included in Supplementary Material 1. Images were acquired from an epifluorescence microscope (Olympus BX61 laboratory microscope, Melville, NY-Olympus America Inc.). The number of SOD1-positive cells was quantified in the hilus of the *dentate gyrus* of the hippocampus.

Real-time polymerase chain reaction

Gene expression was quantified after hippocampal RNA extraction. Posteriorly, RNA samples were retrotranscribed into cDNA and used for real-time polymerase chain reaction (RT-PCR) [26]. Specific protocol details are described in a

previous publication from our research group. Primer sequences for the RT-PCR are in Supplementary Material 2.

Citrate Synthase Activity Colorimetric Assay Kit

Activity was detected from tissue homogenates as described in the protocol by BioVision, Inc. (Citrate Synthase Activity Colorimetric Assay Kit, K318) and corrected for sample-protein content through a Pierce™ BCA Protein Assay Kit (Thermo Scientific™, Waltham, MA, USA).

Golgi stain

Protocol was followed as described by the manufacturer ((FD Rapid GolgiStain™ Kit; Cat #PK401; FD Neurotechnologies, Inc.) and images were obtained from a BX61 laboratory microscope (Melville, NY-Olympus America Inc.).

Dendritic spines were quantified as previously described [26]. Briefly, granular neurons from the *dentate gyrus* of the hippocampus were chosen and measurements were performed at least 50 μm from the soma. At minimum, 5 consecutive 10-μm sections were collected and 5 neurons were quantified per animal. Neurons were chosen from those that showed clear staining. No neurons were included in the analyses if their dendritic arborisation crossed paths with those nearby. Four animals per experimental group were used.

Statistical analysis

Results were presented as interleaved boxes and whiskers with each of the obtained values being represented. The box represents the median and the 25th to 75th percentiles in the extremes. Maximum and minimum values were represented as whiskers. All experimental groups were tested through two-way ANOVA and Tukey's. All analyses and graph representations were performed in the program GraphPad Prism for Windows version 6.01 (GraphPad Software, Inc.). Experimenters were blinded to data sets when calculating statistical differences. Only relevant significant values were represented with their corresponding *p* value. In all figures and graph representations, the *f* value and degrees of freedom for ANOVA were included.

Results

Transgenic JNK1 knockout mice show lower body weight, higher insulin sensitivity, and no negative insulin-related or inflammatory alterations after long-term HFD feeding

Evaluation of periphery parameters demonstrated how lack of JNK1 reduced animal body weight and increased responsiveness to insulin when evaluated in the ITT. WT HFD animals

showed significant increases in body weight, as well as increased blood glucose concentrations both in the GTT and in ITT. HFD caused for mild increases in weight and glucose concentrations in the *Jnk1*^{-/-} HFD experimental group, but values were similar to those of WT CT animals (Fig. 1).

On a molecular level, the IR/AKT signalling axis showed a significant increase of the activating phosphorylation rates in the *Jnk1*^{-/-}s when compared with that in WTs. GSK3β auto-phosphorylated inhibitory Ser9 residue showed similar tendencies. Additionally, IDE protein levels were significantly reduced in the WT HFD experimental group versus that in the WT CT. Finally, protein levels for the PTP1B were strongly increased in the WT HFD animals while *Jnk1*^{-/-}s experimental groups showed non-significant, slightly lower values than WT CT (*p* = 0.1143 and *p* = 0.0816 respectively) (Fig. 2a).

Analysis of the profiles of both astrocytes and microglia revealed that cells were more reactive in the WT HFD when compared against the control. Reactiveness was evaluated regarding the size, colour intensity, number, and ramification rates of the detected cells. In the *Jnk1*^{-/-} experimental groups, a reduction in these same characteristics was observed even below control levels (Fig. 2b).

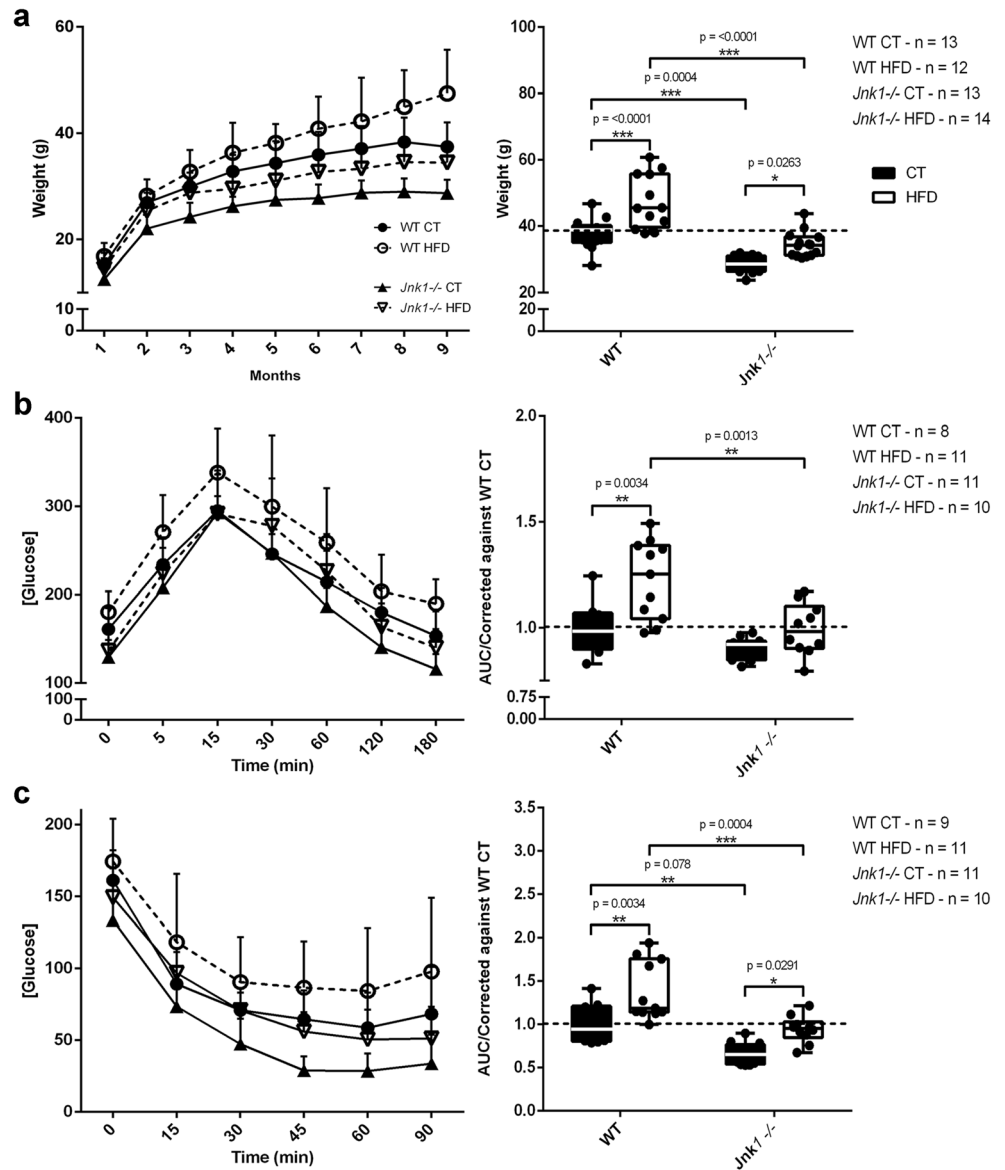
Lack of JNK1 increases mitochondrial oxidative phosphorylation and antioxidant enzymes and protects against HFD-induced dysregulations

Detection of OXPHOS complexes showed an overall tendency towards higher protein levels in the *Jnk1*^{-/-} animals, especially when comparing against the WT HFD mice, which present significant reductions on CI and CII versus WT CT. Exceptionally, CII was strongly affected by HFD even in the *Jnk1*^{-/-} HFD experimental group. Importantly, CIII is highly upregulated in both *Jnk1*^{-/-} experimental groups. Similar upward tendencies were observed in PGC1α and PPARγ, as well as in antioxidant enzymes SOD1 and GPX1 (Fig. 3a). Evaluation of gene expression rates for *Pgc1α*, *Pparγ*, *Sod1*, and *Gpx1* showed a significant upregulation in the *Jnk1*^{-/-} animals (Fig. 3b). Additionally, quantification of the number of SOD-positive cells in the hilus region of the *dentate gyrus* of the hippocampus showed the same trends (Fig. 4a, b). Finally, 4HNE levels were significantly increased in WT HFD and reduced in *Jnk1*^{-/-}s (Fig. 3) and the activity of citrate synthase was mildly higher in the *Jnk1*^{-/-} experimental groups (Fig. 4c).

Absence of JNK1 increases motor activity and protects against cognitive impairment by maintaining dendritic spines and synapse-related proteins even after chronic exposure to HFD

During the habituation period, three parameters related to motor activity were quantified: time spent in the open-field inner quadrant, total distance, and mean speed. In all three

Fig. 1 Periphery metabolic parameters: **a** weight: WT CT = 13, WT HFD = 12, *Jnk1*^{-/-} CT = 13, *Jnk1*^{-/-} HFD = 14; **b** GTT: WT CT = 8, WT HFD = 11, *Jnk1*^{-/-} CT = 11, *Jnk1*^{-/-} HFD = 10; **c** ITT: WT CT = 9, WT HFD = 11, *Jnk1*^{-/-} CT = 11, *Jnk1*^{-/-} HFD = 10. [x]/time progression curves are presented as the mean and standard deviation. Results are presented as interleaved boxes and whiskers. The box represents the median in the middle and the 25th to 75th percentile in the extremes. The maximum and minimum values were represented as whiskers. Statistical analysis: two-way ANOVA and Tukey's (***p* < 0.01 and ****p* < 0.001)



measures, *Jnk1*^{-/-} animals showed higher values than their controls (Fig. 5a–c). Assessment of long-term memory consolidation through the novel object recognition test (NORT) determined that only WT HFD animals had reductions in the discrimination ratio index (Fig. 5d).

Detection of the protein levels of BDNF demonstrated a significant increase in the *Jnk1*^{-/-} mice. Synapse-related proteins ARC, neurexin 2, and neuroligin 3 showed tendencies towards an increase in animals lacking JNK1 (Fig. 5e). Additionally, significant reductions were observed for the WT HFD experimental group versus WT CT in synaptophysin, neurexin 2, and neuroligin 3 protein levels while *Jnk1*^{-/-} HFD showed no diet-induced effects (Fig. 5e). Finally, alterations on neurexin 2 and neuroligin 3 were confirmed in an immunofluorescence detection in the *cornu ammonis* 3 region of the hippocampus (Fig. 6).

Moreover, spinophilin, P-Pyk2, and DBN1, found in dendritic arborisations and spines, showed similar upward tendencies in *Jnk1*^{-/-}s (Fig. 5e), which were correlated with the values in the PAK1/LIMK1 axis (Fig. 7a). Similarly, quantification on the number of dendritic spines showed clear reductions in the WT HFD experimental group while *Jnk1*^{-/-}s showed no differences against WT CT (Fig. 7b). Furthermore, WT HFD spines showed smaller and shorter profiles.

Discussion

The results from the present investigation demonstrate, for the first time, the role of JNK1 in the context of the appearance of cognitive deficits linked to metabolic alterations [28].

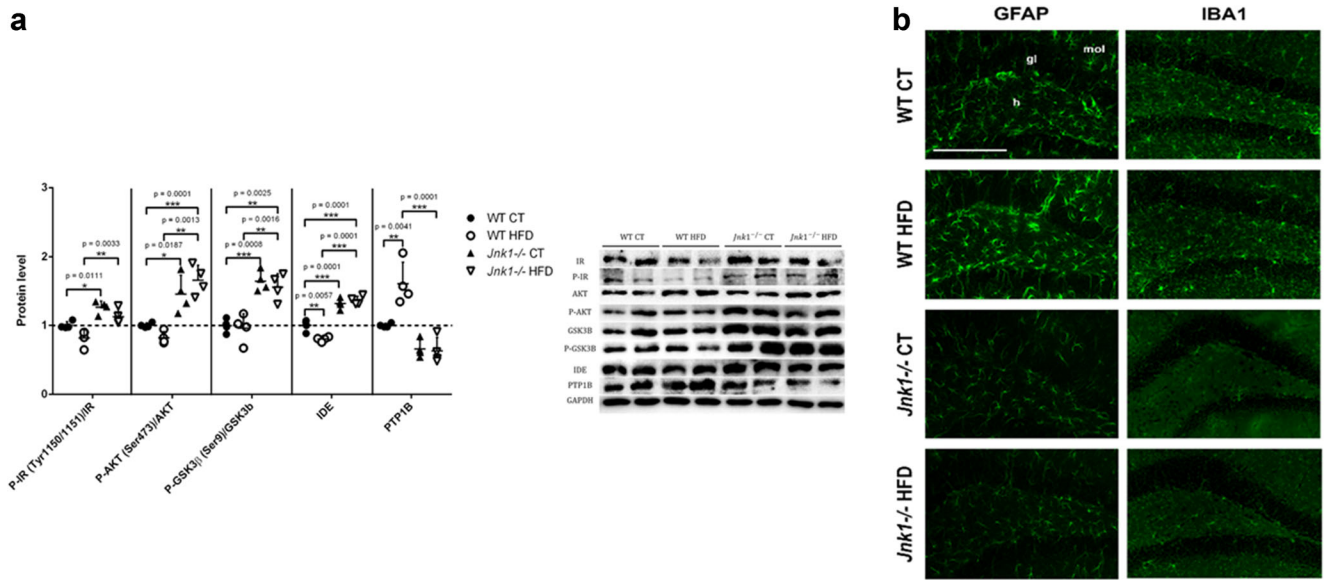


Fig. 2 a Evaluation of biomarkers associated with the cellular signalling of insulin through the detection of protein levels. Results are presented as scatter plots representing individual values. Mean ± SD. *n* = 4. Statistical analysis: two-way ANOVA and Tukey's (**p* < 0.05, ***p* < 0.01, and

****p* < 0.001). **b** Analysis on the reactive profiles of astrocytes (GFAP; green; first column) and microglia (IBA1; green; second column) in the dentate gyrus of the hippocampus. Representative images were presented. Scale bar 200 μm. mol, molecular layer; gl, granular layer; h, hilus

The appearance of hyperglycaemia and loss of insulin sensitivity in the periphery and central tissues has been

previously reported in models of HFD both by us and by other research groups [26, 29]. It is believed that this

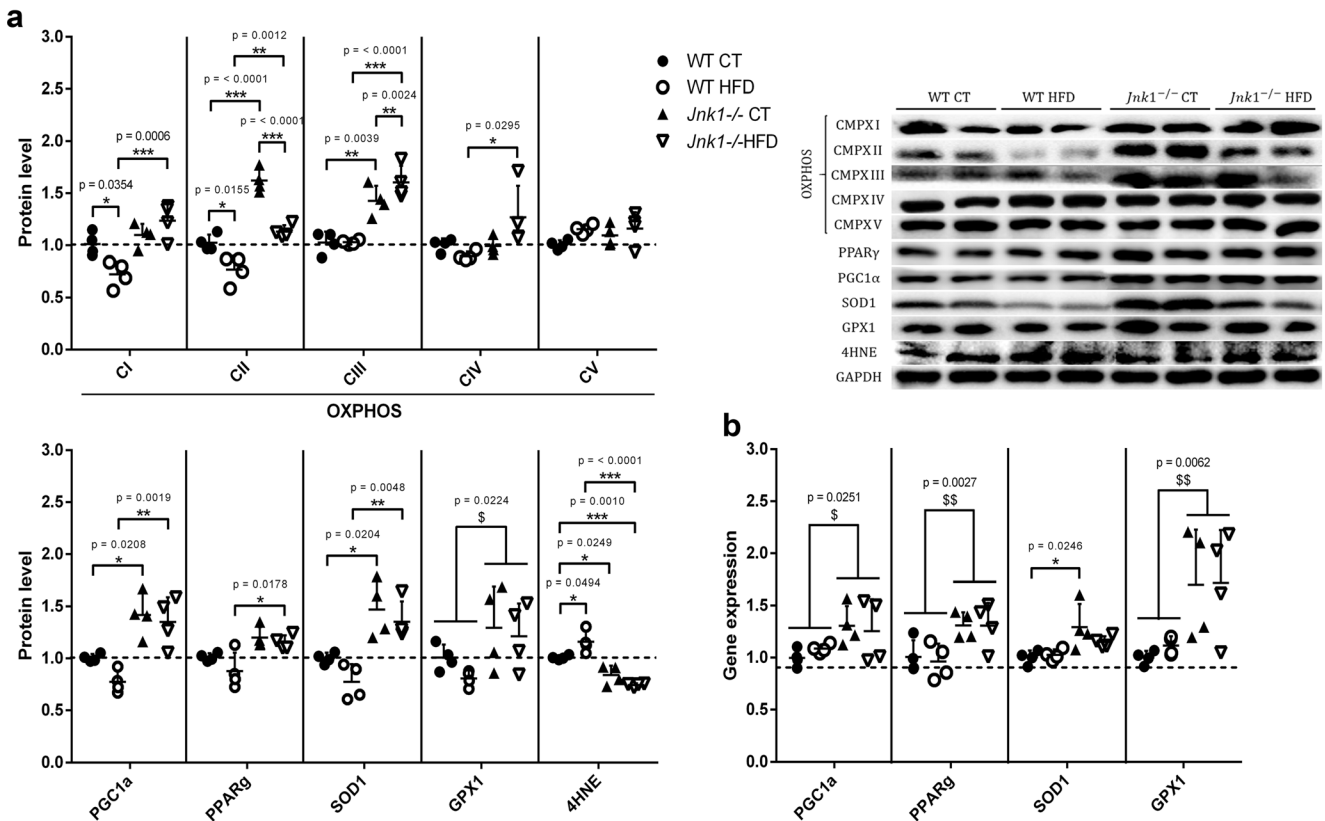


Fig. 3 Detection of mitochondrial oxidative phosphorylation complexes and antioxidant enzymes through **a** protein levels and **b** gene expression. Results are presented as scatter plots representing individual values. Mean

± SD. *n* = 4. Statistical analysis: two-way ANOVA (^{\$}*p* < 0.05 and ^{\$\$}*p* < 0.01) and Tukey's (**p* < 0.05, ***p* < 0.01, and ****p* < 0.001)

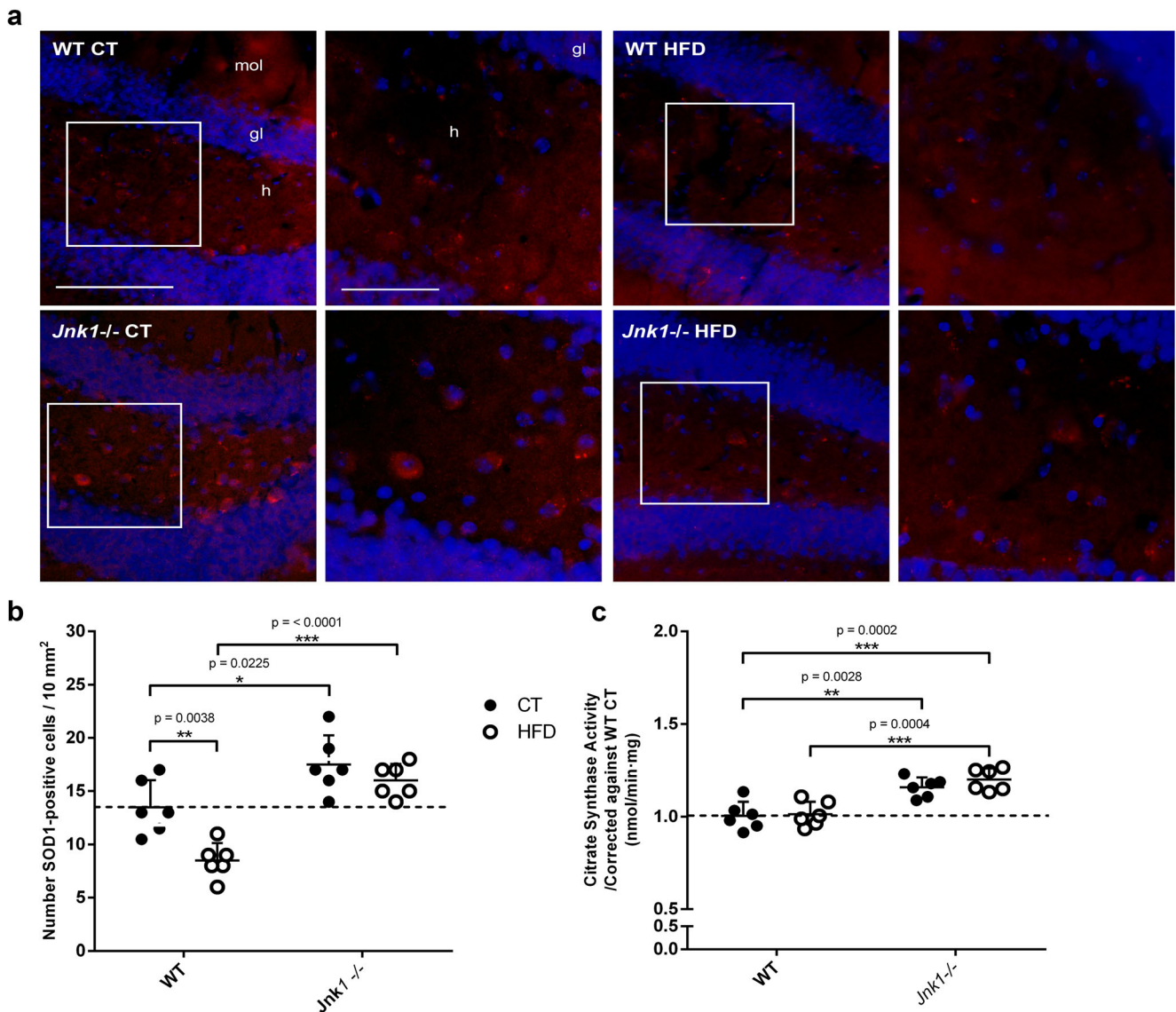


Fig. 4 a Representative images of labelling against SOD1 (red) in the dentate gyrus of the hippocampus. Cellular nuclei were stained with Hoechst (blue). Scale bar (1) 200 μm and (2) 100 μm. mol, molecular layer; gl, granular layer; h, hilus. **b** Quantification of the number of

SOD1-positive cells/10 mm². *n* = 6. **c** Results of the citrate synthase activity assay. *n* = 6. Results are presented as scatter plots representing individual values. Mean ± SD. Statistical analysis: two-way ANOVA and Tukey's (**p* < 0.05, ***p* < 0.01, and ****p* < 0.001)

situation derives from the development of mitochondrial and endoplasmic reticulum stress which in turn increases the activity of the JNKs, kinases responsible for the inhibitory phosphorylation of the IRS1. Moreover, other molecules like PTP1B and SOCS3, which are phosphatases of the tyrosine activation points, also become upregulated [30–32]. These mechanisms were confirmed in a previous publication from our group in which the effects of the removal of JNK2 were studied [26]. Additionally, other alterations like the increased production of ceramides favour the appearance of metabolic dysfunctions, impairments of neuronal plasticity, reduced myelin maintenance, glial and neuronal cell death, neurodegeneration, and cognitive affectations [33].

Alternatively, modulation of the activity of the JNK1 seems like a promising approach to reverse this situation. As it has been summarized in “Introduction,” the whole-body inactivation of JNK1 or the neuron-specific silencing of this kinase causes mice to become significantly more sensitive to insulin even when fed chronically with HFD [21, 23]. In the brain, when analyzing hippocampal extracts, significant increases in the phosphorylation rates of the IR, AKT, and GSK3β proteins were observed when comparing against the control, thus indicating higher activation of the IR axis. Thus, higher activity of the insulin signalling pathway after the negative modulation of JNK1 favours the maintenance of glucose homeostasis and increases cell survival and activity, allowing for the protection of brain function against metabolic diseases.

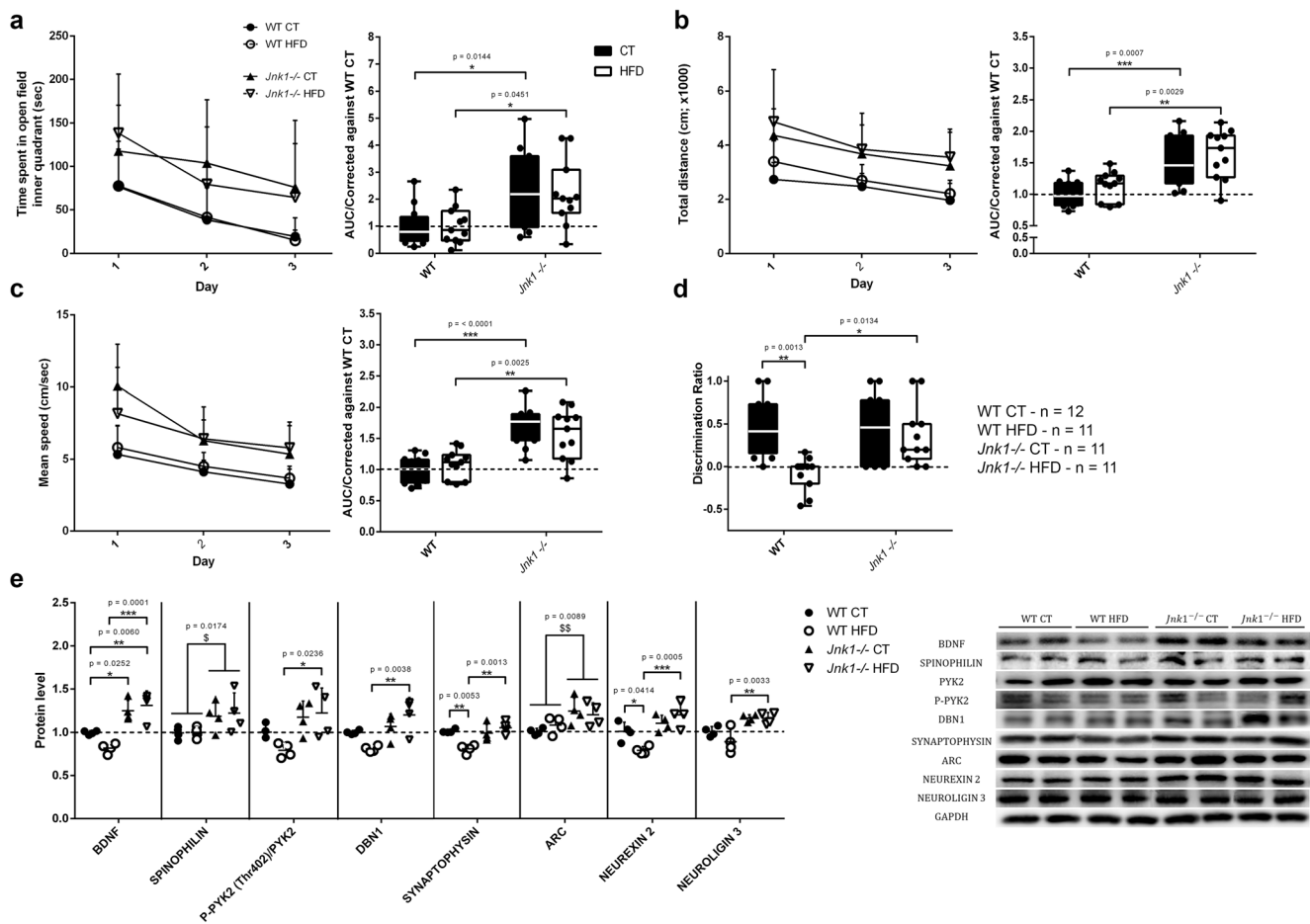


Fig. 5 Behavioural assessment through NORT in an open field. **a** Time spent in open-field inner quadrant (s), **b** total distance (cm; $\times 1000$), **c** mean speed (cm/s), and **d** discrimination ratio. The first three measurements (**a–c**) were quantified during the habituation period. WT CT = 12, WT HFD = 11, *Jnk1*^{-/-} CT = 11, *Jnk1*^{-/-} HFD = 11; [\bar{x}]/time progression curves are presented as the mean and standard deviation. Results are presented as interleaved boxes and whiskers. The box represents the

median in the middle and the 25th to 75th percentile in the extremes. The maximum and minimum values were represented as whiskers. **e** Protein-level detection against BDNF, spinophilin, P-PYK2(Thr402)/PYK2, DBN1, synaptophysin, ARC, neuexin 2, and neuroligin 3. Results are presented as scatter plots representing individual values. Mean \pm SD. Statistical analysis: two-way ANOVA and Tukey's (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$)

When evaluating the state of mitochondria, a reduction in oxidative capacity was observed in WT HFD mice. This conclusion was drawn from the detection of a decrease in the levels of the OXPHOS complexes, levels and expression of antioxidant enzymes, and PGC1 α and PPAR γ agents. Similar observations in other tissues have already been reported by other researchers [34] which have described a relationship between impairments in PGC1 α and the appearance of cognitive affectations through the downregulation of BDNF [35]. Furthermore, evidence suggests that PPAR γ has protective roles through the regulation of SOD activity [36], and consequently, its alteration might participate in mitochondrial dysregulation. Importantly, animals that lacked JNK1 showed increased mitochondrial function, even when detecting the activity of citrate synthase enzyme, a component of the Krebs cycle. So, higher mitochondrial activity will account for increased energy expenditure. This conclusion, together with the fact that JNK1 is involved with anxiety behaviours

and other mechanisms like the maintenance of reservoirs in the adipose tissue, and the observed increased motor activity of the *Jnk1*^{-/-}, could account for the reduction in the total body weight of these animals. *Jnk1*^{-/-} HFD animals showed high resilience to the negative effects of HFD in the mitochondria, thus indicating the protective potential derived from the inhibition of this kinase.

As a result of metabolic affectations, there is also an increased release of molecules like cytokines (TNF α , IL6, etc.) and eicosanoids by adipocytes [37, 38], which induce inflammatory responses. In the brain, astrocytes and microglia respond strongly to those molecules [39]. The appearance of chronic inflammatory reactions in the brain leads to degeneration due to the induction of apoptotic mechanisms in neurons and other neural types [40, 41]. It has been described that HFD, as a method to cause environmentally induced obesity in preclinical models, induces increased cellular reactivity [26, 42]. In our study, these same observations were made and,

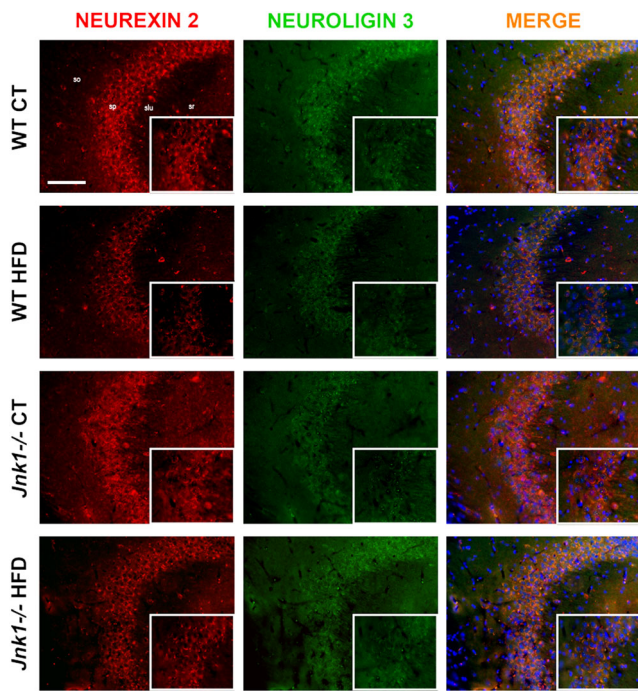


Fig. 6 Distribution of neurexin 2 (red, presynaptic protein) and neuroligin 3 (green, postsynaptic protein) in the *cornu ammonis 3* area of the hippocampus. Cellular nuclei are stained with Hoechst (blue). Scale bar 200 μm . so, *stratum oriens*; sp, pyramidal layer; slu, *stratum lucidum* and *stratum radiatum*

most significantly, it was clear that *Jnk1*^{-/-} animals presented qualitatively lower reactivity even against the control group. This result is in accordance with the relevance that JNK1 has in the activation of these cellular types [43–49].

In the end, the metabolic alterations caused by the HFD lead to the appearance of cognitive impairments [44]. Our results have shown clear affectations in the capacity of

animals to generate long-term memory as assessed by the NORT. Furthermore, the analysis of proteins like synaptophysin and neurexin related to the establishment of synaptic connections supported these assumptions, together with the reduction in the number and size of dendritic spines. Similar tendencies towards a reduction in the levels of other molecules like BDNF or Drebrin were observed, but statistical analyses deem them non-significant. Nonetheless, reported bibliography has already described the negative effects of dysregulation of metabolism in cognitive function [3]. Importantly, *Jnk1*^{-/-} experimental animals showed evidence of the protective and beneficial effects of the modulation of the activity of this kinase. These experimental groups had normal cognitive capacity in the NORT and, in some cases, showed higher levels for proteins related to the maintenance of synapses and dendritic spine density. Especially relevant results were observed in the high upregulation of BDNF, the maintenance of neurexin and neuroligin proteins, and the increased activation of PAK1 and LIMK1 proteins, responsible of the inhibition of Cofilin, a known destabilizing element of cytoskeletal microfilaments of the structure of dendritic spines [45].

In conclusion, metabolic dysregulations and posterior cognitive impairments are prevented when negatively modulating the activity of JNK1. Therefore, it is of interest to consider this target for the design of future strategies to treat these pathologies, taking into account that the use of a partial, pharmacological approach will most likely avoid the reported skin oxidative damage in the whole-body knockout animals. Additionally, the use of a molecule derived from natural products like licochalcone A (JNK1 inhibitor), a compound already tested by our research group in a model of temporal lobe epilepsy [46], will prove to cause less secondary side effects

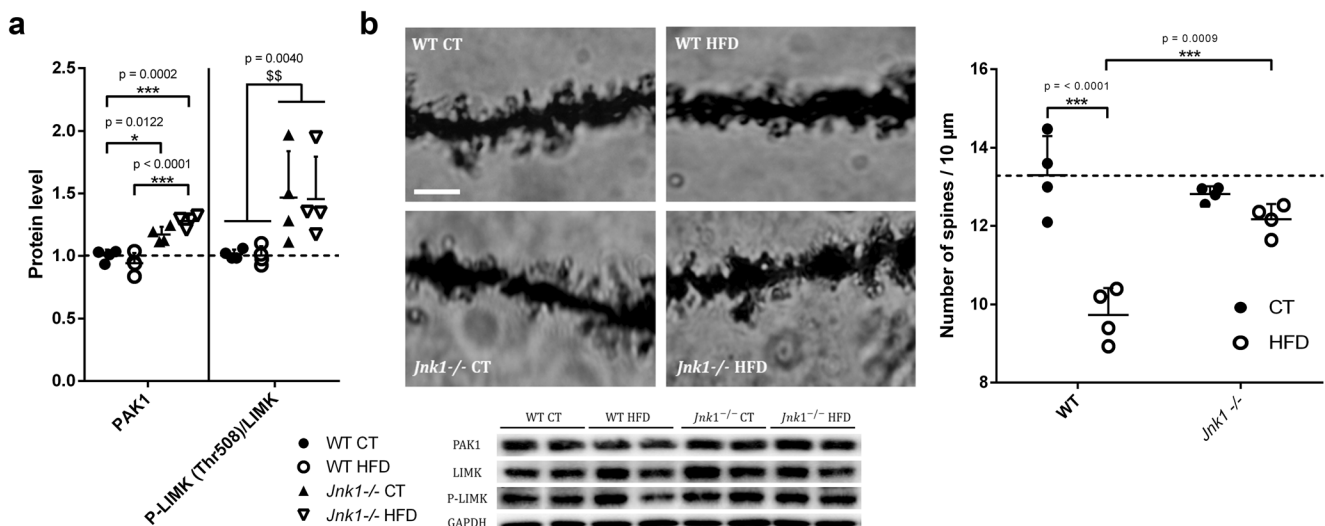


Fig. 7 **a** Determination of the levels for the PAK1 and LIMK protein axis. **b** Representative images and quantification of the number of dendritic spines for each of the experimental groups. Scale bar 5 μm . Results are

presented as scatter plots representing individual values. Mean \pm SD. $n = 4$. Statistical analysis: two-way ANOVA (^{\$} $p < 0.01$) and Tukey’s (^{*} $p < 0.05$ and ^{***} $p < 0.001$)

than those typically caused by the chronic use of synthetic drugs.

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Authors' contribution OB was the lead scientist of this research; he worked on the experimental design, procedures, and the manuscript. AE, JO, RDCT, and TEJ contributed on the experiments. ME took part in the experimental design and experimental trouble solving. EV, AC, JF, and CA provided the first animals to create the colony, helped with the experimental design, and corrected the manuscript. JF, GC, CBZ, MB, and AC provided ideas and helped write and correct the manuscript.

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Compliance with ethical standards

During all procedures, the European Committee bioethics directives were followed (European Communities Council Directive 2010/63/EU) and all protocols were previously approved by the ethics committee from the University of Barcelona

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