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Thidoredxin-2 overexpression fails to rescue chronic high calorie diet induced hippocampal dysfunction

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Abstract

A high calorie diet (HCD) diet can impair hippocampal synaptic plasticity and cognitive function in animal models. Mitochondrial thioredoxin 2 (TRX-2) is critical for maintaining intracellular redox status, but whether it can protect against HCD-induced impairment of synaptic plasticity is unknown. We found that levels of TRX-2 are reduced in the hippocampus of wild type mice maintained for 8 months on a HCD, and that the mice on the HCD exhibit impaired hippocampal synaptic plasticity (long-term potentiation at CA1 synapses) and cognitive function (novel object recognition). Transgenic mice overexpressing human TRX-2 (hTRX-2) exhibit increased resistance to diquat-induced oxidative stress in peripheral tissues. However, neither the HCD nor hTRX-2 overexpression affected levels of lipid peroxidation products (F2 isoprostanes) in the hippocampus, and hTRX-2 transgenic mice were not protected against the adverse effects of the HCD on hippocampal synaptic plasticity and cognitive function. Our findings indicate that TRX-2 overexpression does not mitigate adverse effects of a HCD on synaptic plasticity, and also suggest that oxidative stress may not be a pivotal factor in the impairment of synaptic plasticity and cognitive function caused by HCDs.

Keywords

high calorie diet; hippocampus; lipid peroxidation; mitochondria; oxidative stress; synaptic plasticity; thioredoxin

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Introduction

The chronic positive energy balance responsible for the ongoing epidemic of obesity and consequent diabetes and cardiovascular disease, can also have a negative impact on cognitive function and may increase the risk of dementia (Elias et al. 2005; White et al. 2009; Kanoski and Davidson, 2011; Mattson, 2012; Yau et al., 2012; De Felice and Ferreira, 2014). Electrophysiological analyses in rodent models have shown that the adverse effects of high calorie diets (HCDs) on learning and memory are associated with impaired hippocampal synaptic plasticity (Farr et al., 2008; Stranahan et al., 2008a). A HCD (typically high fat, and glucose or fructose) can cause oxidative damage to proteins, and lipid peroxidation in muscle, liver, kidney and brain (Bonnard et al. 2008; Matsuzawa-Nagata et al. 2008; Stranahan et al., 2011; White et al. 2009; Ruggiero et al. 2011). Mitochondrial and plasma membrane-associated oxidative stress are associated with cognitive deficits in human subjects with Alzheimer's disease (Sultana and Butterfield, 2009; Du et al., 2012), and are sufficient to impair hippocampal synaptic plasticity and cause cognitive deficits in experimental models (Mattson, 2004; Serrano and Klann, 2004; Freeman et al., 2014). Moreover, high and low glycemic index diets increase and decrease cerebrospinal fluid markers of oxidative stress in human subjects with mild cognitive impairment (Bayer-Carter et al., 2011). Collectively, findings suggest that oxidative stress may play a crucial role in mediating adverse effects of HCDs on cognitive function.

Mitochondria are the source of the majority of reactive oxygen species (ROS) generated in cells, with ROS production occurring at particularly high levels in excitable cells such as neurons (Mattson et al., 2008). Superoxide anion radical is generated at mitochondrial inner membrane electron transport chain protein complexes I and III during oxidative phosphorylation, and is then converted to hydrogen peroxide by superoxide dismutases in the mitochondria (SOD2) and cytoplasm (SOD1). In the presence of Fe^{2+} or Cu^{+}, hydroxyl radical is generated from hydrogen peroxide. Hydroxyl radical and peroxynitrite (produced by the interaction of superoxide with nitric oxide) can induce membrane lipid peroxidation, protein oxidation and DNA damage. Hydrogen peroxide levels are normally kept low by its catabolism by catalase and glutathione peroxidases. In addition to the enzymes that directly detoxify ROS, the thioredoxin system, comprised of thioredoxin (TRX), TRX reductase and NADPH, is critical for maintaining cellular redox balance and cellular antioxidant robustness (Lu and Holmgren 2012). Thioredoxin 2 (TRX-2) is a protein disulfide oxidoreductase located in mitochondria where it plays an antioxidant role by transferring electrons to peroxiredoxins and methionine sulfoxide reductases. TRX-2 deficiency results in elevated ROS levels and increased vulnerability to apoptosis in cultured immune cells (Tanaka et al., 2002). In addition, mice in which the *Trx-2* gene is selectively knocked out out in cardiac myocytes exhibit a cardiomyopathy associated with mitochondrial oxidative stress, membrane depolarization and a cellular ATP deficit (Huang et al., 2015). It has also been reported that overexpression of TRX-1 and TRX-2 can protect retinal ganglion neurons against degeneration in experimental models relevant to macular degeneration and glaucoma (Caprioli et al., 2009).

The major excitatory synapses in the mammalian brain deploy glutamate as a neurotransmitter. Activation of postsynaptic glutamate receptors results in membrane

depolarization and Ca^{2+} influx which initiates signaling cascades involving kinases and transcription factors that mediate the adaptive responses in synaptic function and structure that are involved in learning and memory (Nicoll and Roche, 2013). Activation of glutamatergic synapses results in increased mitochondrial electron transport chain activity to generate the ATP required to sustain the function of ion-motive ATPases. In addition, Ca^{2+} uptake by mitochondria promotes ROS production in neurons stimulated by glutamate (Duchen, 2000; Pivovarova and Andrews, 2010). Whereas under normal circumstances neurons are able to mitigate the oxidative stress resulting from synaptic activity, excessive energy intake and obesity may compromise antioxidant defenses in neurons. Because TRX2 would be expected to counteract adverse effects of excessive energy intake on synaptic plasticity, we employed human *Trx-2* transgenic (hTRX-2) mice to determine whether overexpression of TRX-2 can rescue high-fat diet-induced hippocampal synaptic dysfunction and cognitive impairment.

Methods

Generation and genotyping of *Trx-2* transgenic mice

Founder mice in which the human *Trx-2* gene (GenBank: NC_000022) was expressed using regulatory elements from the human β-actin promotor were created by Xenogene Biosciences Laboratories. A 3702 bp microinjection fragment (MIF) containing the human *Trx-2* cDNA was isolated from the plasmid (Invitrogen pDRIVE03-β-actin(h) V02) by *SpeI* and *SwaI* restriction enzyme digestion. The MIF was injected into fertilized mouse eggs from C57BL/6 donors, and implanted into pseudopregnant females. Chimeric pups were bred to generate heterozygous offspring. Mice were maintained on a 12 hour light/dark cycle with free access to food and water. All procedures were approved by the Animal Care and Use Committee of the National Institute on Aging and were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Male hTRX-2 mice were bred to C56BL/6J female mice to generate offspring for these studies. Male mice were used for all experiments.

One month-old mice were genotyped by assaying for the presence of the mitochondrial *hTrx-2* sequence by polymerase chain reaction (PCR). Genomic DNA was extracted from mouse tail snips by standard protocols. Briefly, tail snips were placed in DirectPCR Lysis Reagent (tail) (Viagen Biotech.Inc., cat# 101-T) containing 0.3 mg/ml Proteinase K (Sigma, Cat# p6556) and incubated overnight at 65 °C until complete lysis was achieved; DNA was centrifuged (7,500 x g for 5 min) and the supernatant was transferred to a clean 1.5 ml Eppendorf tube. A PCR amplification was performed in a 25 μl reaction using 1 μl of DNA lysate, with the following primers: *hTrx-2* specific forward primer 0.1 μl (20 μM) (5′-OH CCAATCTGAAACCTCGTGC OH-3′); *hTrx-2* specific reverse primer 0.1 μl (20 μM) (5′–OH AAACCTCCCACACCTCCC OH-3′), and 10 μl of Promega GoTaq Green Master Kit solution (Promega, cat# M7122). The mixture was subjected to the following PCR conditions: initial denaturation at 95°C for 2 minutes; 35 repeated cycles of denaturation at 95°C for 50 s; primer annealing at 58°C for 50 s and elongation at 72°C for 60 s; and a final extension cycle of 72°C for 5 min. Subsequently, 25 μl of the amplification reaction was

analyzed on a 1.2% agarose – ethidium bromide gel to establish the presence or absence of a 360 bp PCR product indicative of the presence of the human *Trx-2* gene.

Diets

Beginning at 5 months of age, mice were maintained for 8 months on either standard chow or a HCD. Mice on the HCD were fed a high fat diet purchased from Dyets Inc. (diet #101842) and were provided drinking water containing 11% of a 1:1 mixture of fructose and glucose.

Immunoblot analysis

Hippocampal tissue was homogenized in RIPA buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 2.5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 1% sodium deoxycholate) containing protease inhibitors. The amount of total proteins in the homogenates was estimated with a Pierce BCA protein assay kit (Pierce Biotechnology). Protein samples were separated in a 4–20% acrylamide gradient gel and transferred to a nitrocellulose membrane. Following incubation with blocking buffer (5% dry milk and 0.05% Tween20 in PBS), the membranes were incubated overnight at 4°C in the blocking buffer containing a 1:800 dilution of TRX-2 rabbit polyclonal antibody (Cell Signaling Technology, #13322). The amino acid sequence of human TRX2 is 98% identical to mouse TRX2. The antibody used in the present study was generated against a synthetic peptide with an amino acid sequence identical to a conserved sequence in the carboxyl terminus of TRX2. The membrane was then washed (0.1% Tween20 in PBS) and incubated with peroxidase-conjugated anti-rabbit secondary antibody. The proteins were visualized using a chemiluminescence kit from Pierce. The intensities of protein bands were analyzed using ImageJ software.

Diquat treatment and F_2-isoprostane assay

Groups of TRX-2 transgenic mice and their wild type littermates (6–8 months of age) were injected intraperitoneally with diquat dissolved in saline and administered at a dose of 50 mg/kg. Three and six hours after injection, the mice were sacrificed and liver tissue and blood were taken for assay. F_2-isoprostanes were measured using gas chromatography-mass spectrometry methods as described previously (Morrow and Roberts, 1994). The isoprostane levels were measured in samples of 100 mg wet weight of tissue or 1 ml of plasma. Tissue was homogenized in chloroform:methanol containing BHT (0.005%) to prevent auto-oxidation, dried under a stream of nitrogen, and re-suspended in methanol containing BHT. Esterified F_2-isoprostanes in phospholipids were saponified by adding aqueous potassium hydroxide (this frees fatty acids from lipids). The sample was acidified and diluted with water. Deuterated-F_2-isoprostane internal standard was then added to the mixture. For the measurement of free F_2-isoprotanes in plasma, the extraction and hydrolysis steps were omitted, and the sample was simply acidified, diluted, and the internal standard added. The mixture was subsequently run on a silica column to separate isoprostanes from bulk fatty acids. The eluate was converted to pentafluorobenzyl esters, by treatment with pentafluorobenzyl bromide (this step was necessary because free fatty acids are difficult to separate by gas chromatography). The mixture was subjected to thin layer chromatography

to remove the excess pentafluorobenzyl bromide and unreacted fatty acids. The F_2-isoprotane/isofurane fraction was extracted using ethyl acetate, and analyzed by injection into a Thermo Finnigan TRACE DSQ single quadrupole mass spectrometer. This instrument is capable of electron impact and chemical ionization, with positive and negative ion detection. The F_2-isoprostanes were quantified by peak height, and the data were corrected with the internal standard and expressed as nanograms of F_2-isoprostanes per mL of plasma or per gram tissue.

Hippocampal slice electrophysiology

These methods were similar to those described previously (Wang et al., 2009; Zhang et al., 2011). Briefly, Transverse hippocampal slices (350 μm) were maintained in oxygenated artificial cerebrospinal fluid (ACSF) containing (mM): 120 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1.3 $MgSO_4$, 2.5 $CaCl_2$ and 10 glucose (pH 7.4; osmolality was 290 mmol/kg). Field potential recordings were performed at 30–32°C in a perfusion chamber. For LTP experiments, 50 μM picrotoxin was added in ACSF to block $GABA_A$ receptors. Only slices in which field recordings exhibited a steep input-output curve were included. Excitatory postsynaptic potentials (EPSPs) were recorded in CA1 stratum radiatum with stimuli (30 μs duration every 20 s) delivered with a bipolar tungsten electrode to activate Schaffer collateral/commissural afferents. LTP was induced with a train of tetanic stimulation (100 Hz for 1s). Plots were normalized to the initial slope of the EPSPs with each data point representing the averaged values for 1 min (three consecutive sweeps with an interval of 20 sec). Data were collected using an Axopatch 1D amplifier (Molecular Device); signals were filtered at 2 kHz, digitized at 10 kHz and analyzed using pCLAMP 8 software (Molecular Devices).

Behavioral tests

These methods have been described previously (Okun et al., 2010). Briefly, for the novel object recognition test the mice were habituated to the experimental room and 25 cm × 25 cm opaque walled test cages for 3 consecutive days, 30 minutes each time and for 1 hour on the testing day. These experiments were conducted under a light intensity of 15 lux. Mice were first allowed to explore their cage in the presence of two identical objects and were then returned to their home cages for 10 minutes (short-term memory). The mice were then placed in the test cage with one familiar object and a novel object for 150 seconds. Twenty four hours later (long-term memory) the mice were placed in the test cage with one familiar object and a novel object for 150 seconds. Object preferences were automatically analyzed using Anymaze video tracking software. An object preference index was determined by calculating the time spent near the novel object divided by the cumulative time spent near both objects. For open field testing mice were placed in the center of an open field (MED-OFA-MS system; Med-associates, St. Albans, VT, USA) under a light intensity of 400 lux and exploration was assessed for 15 minutes. Cages were cleaned with ethanol following each session. The open field was 40 cm × 40 cm, and the peripheral 10 cm was designated as the peripheral zone and the central 30 cm^2 was designateds the central zone.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) with Bonferroni post hoc analysis were performed using Graphpad Prism 5.0 (La Jolla, CA, USA) with significance set at $p < 0.05$.

Results

Prior to diet initiation (5 month-old mice) the body weights of all mice were in the range of 32–38 grams. Wild type and TRX-2 transgenic mice on the control diet gained only about 5 grams of weight over the 8-month period (Figure 1A). In contrast, wild type and TRX-2 transgenic mice on the HCD gained more than 20 grams during an 8-month period. There were no significant differences in body weights of wild type and TRX-2 transgenic mice at any time point (Figure 1A). To determine whether overexpression of TRX-2 modifies cellular oxidative stress we measured levels of F_2-isoprostanes in liver tissue and plasma taken from mice that had been exposed to diaquat (50 mg/kg i.p.) and then euthanized at three time points (0, 3 and 6 hours). F_2-isoprostanes are produced by free radical attack on arachidonic acid in situ in membrane phospholipids, and are chemically stable end-products of lipid peroxidation. F2-isoprostane levels were significantly lower in the plasma at the 3 hour time point, and in the liver at the 3 and 6 hour time points, in TRX-2 transgenic mice compared to wild type mice (Figure 1B). These finding suggest that TRX-2 overexpression increases the resistance of mice to diquat-induced oxidative stress.

We next focused on cohorts of wild type and TRX-2 transgenic mice that had been maintained on either a control diet or a HCD for 8 months (8–10 mice/group). To determine whether chronic HCD affects levels of oxidative stress, and to determine whether overexpression of TRX-2 modifies such oxidative stress, we measures levels of F_2-isoprostanes in liver tissue and brain tissue of mice that had been maintained on the control diet or a HFD. F2-isoprostane levels were significantly higher in the liver of wild type mice on the HCD compared with those on the control diet. In contrast, the HCD did not affect levels of F2-isoprostanes in the liver of TRX-2 transgenic mice (Figure 1C, upper graph). Interestingly, whereas the HCD resulted in elevated levels of F2-isoprostane in the liver, the HCD had no significant effect on F2-isoprostane levels in brain tissue (Figure 1C). These finding suggest that TRX-2 overexpression increases the resistance of mice to chronic HCD-induced oxidative stress in peripheral organs, whereas the brain appears relatively resistant to HCD-induced increases of lipid peroxidation.

To confirm that levels of TRX-2 protein were elevated in brain cells of TRX-2 transgenic mice, we performed immunoblot analysis of TRX-2 in hippocampal tissue samples from wild type and TRX-2 transgenic mice that had been maintained on the control diet or HCD for 8 months. Levels of TRX-2 were 40–60% higher in hippocampus of TRX-2 transgenic mice regardless of diet (Figure 1D). Interestingly, in wild-type mice hippocampal TRX-2 levels were significantly lower in mice in the HCD group compared to those on the control diet group (Figure 1D), suggesting that the HCD results in down-regulation of expression of the endogenous mouse TRX-2.

To determine whether TRX-2 overexpression can rescue the impaired synaptic plasticity caused by a HCD, field excitatory postsynaptic potentials (EPSP) were recorded in stratum radiatum of the CA1 region of the hippocampus from wild type and TRX-2 transgenic mice that had been maintained for 8 months on either the control diet or the HCD. Wild type mice on the HCD exhibited impaired LTP compared to wild type mice on the control diet, whereas short-term potentiation (0–10 minutes post tetanus) was unaffected by diet (Figure 2A, B, D). TRX-2 transgenic mice on the control diet exhibited LTP that was similar to wild type mice on the control diet (Figure 2C). LTP was significantly impaired by the HCD in TRX-2 transgenic mice by an amount similar to that of wild type mice on the HCD (Figure 2C, D). We also evaluated basal synaptic transmission by generating input-output curves (Figure 2E), and presynaptic neurotransmitter release by measuring the paired-pulse ratio with increasing interstimulus intervals (Figure 2F). No significant differences were found among the four groups. Collectively, the results of our electrophysiological data indicate that postsynaptic dysfunction mediates the impairment of hippocampal synaptic plasticity caused by a HCD, and that this diet-induced deficit is not ameliorated by TRX-2 overexpression.

It was previously reported that a HCD impairs hippocampus-dependent learning and memory in rats (Konoski et al., 2007; Stranahan et al., 2008; Hsu et al., 2015) and mice (Morrison et al., 2010; Valladolid-Acebes et al., 2013; Arnold et al., 2014). We evaluated hippocampus-dependent learning and memory using a novel object recognition test in wild type and TRX-2 transgenic mice that had been maintained for 8 months on either the control diet or the HCD. After a period of familiarization with two objects in the test cage, mice were removed from the cage for 10 minutes and then returned to the test cage in which one of the objects had been replaced with a novel object (Figure 3A). Wild type and TRX-2 transgenic mice that had been maintained on the control diet spent significantly more time exploring the novel object compared to the familiar object (Figure 3B). In contrast, wild type and TRX-2 transgenic mice on the HCD did not spend more time exploring the novel object (Figure 3B), indicating that the HCD impairs short-term memory and that TRX-2 overexpression does not ameliorate this adverse effect of the HCD on hippocampus-dependent memory.

Anxiety disorders account for considerable morbidity and increase the risk of depression (Whiteford et al., 2013). Obese human subjects are at increased risk of anxiety disorders (Hryhorczuk et al., 2013), and excessive energy intake causes anxiety-like behaviors in rats and mice (Finger et al., 2010; Sharma and Fulton, 2013; Warneke et al., 2014; Sivanathan et al., 2015). We found that mice maintained on the HCD exhibited increased anxiety as indicated by less time spent and distance traveled in the center zone of the open field, with no difference between wild type and TRX-2 transgenic mice (Figure 3C, D). The total distance traveled, time spent moving and time spent rearing were unaffected by genotype or diet, indicating that diet and TRX-2 levels did not alter motor behavior of the mice in the open field test (Figure 3E).

Discussion

TRX-2 is critical for survival as TRX-2−/− mice die during embryonic development at a stage prior to neural tube closure (E10.5) as a result of massive apoptosis of cells (Nonn, et

al., 2003). Mice with reduced TRX-2 levels exhibit impaired mitochondrial function and increased oxidative stress in many tissues (Perez et al., 2008). Because excessive oxidative stress was previously associated with adverse effects of a HCD on hippocampal plasticity (We et al., 2004; Morrison et al., 2010; Stranahan et al., 2011; Karimi et al., 2013; Tucsek et al., 2014), and because TRX-2 can reduce mitochondrial oxidative stress (Perez et al., 2008; Lowes and Galley, 2011), we tested the hypothesis that overexpression of TRX-2 would counteract the adverse effects of a HCD on hippocampal synaptic plasticity, and learning and memory. We generated mice overexpressing hTRX-2 and found that they exhibited reduced levels of lipid peroxidation products in plasma and liver when challenged with the diquat, an agent that induces redox cycling and superoxide production. Whereas wild type mice maintained on a HCD for 8 months exhibited reduced levels of TRX-2 in the hippocampus, TRX-2 levels were maintained at an elevated level in TRX-2 transgenic mice on the HCD. However, overexpression of TRX-2 did not rescue the deficits in hippocampal LTP and hippocampus-dependent short-term memory caused by the HCD.

We found that mice maintained on a HCD for 8 months exhibited a highly significant impairment of LTP at CA1 synapses. The impaired LTP is apparently the result of a postsynaptic dysfunction as paired-pulse facilitation, a measure of release of glutamate from presynaptic terminals was unaffected by the diet. The input-output curves were also similar in wild type mice on the HCD compared to those on the control diet, suggesting that the HCD did not alter basal synaptic transmission. All mice on the HCD gained weight progressively and by 8 months averaged approximately 60 grams, compared to 38 grams in mice on the control diet. Thus, the mice on the HCD were obese at the time of behavioral and electrophysiological analyses. Previous studies have shown that hippocampal CA1 LTP is impaired in several rodent models of obesity, including leptin receptor mutant mice (Stranahan et al., 2008b), rats expressing insulin receptor antisense in hypothalamic cells (Grillo et al., 2011) and male mice fed a high fat diet (Hwang et al., 2010). We found that the initial enhancement of postsynaptic excitatory responses to high frequency stimulation was similar in hippocampal synapses of mice in the HCD and control diet groups, but that the postsynaptic response in the HCD group rapidly declined towards baseline within 20 minutes, whereas potentiation was sustained for at least 60 minutes in the control diet group. We found no differences in synaptic plasticity in hippocampal slices from wild type and TRX-2 transgenic mice in either the control diet or HCD groups. Because TRX-2 protein levels were reduced by more than 50% in wild type mice on the HCD compared the control diet, and were elevated by 50–70% in hTRX-2 transgenic mice regardless of diet, we conclude that changes in TRX-2 levels across a 3-fold range do not influence plasticity at hippocampal CA1 synapses.

Although previous studies have reported that rats or mice fed a diet with high amounts of fats exhibit increased levels of markers of oxidative stress in their brain cells (Matsuzawa-Nagata et al., 2008; Stranahan et al., 2008a), we found that the level of the lipid peroxidation product F_2-isoprostane was unaffected by the HCD in the present study. Although TRX-2 deficiency results in increased oxidative stress and can render cells vulnerable to apoptosis (Nonn et al., 2003; Perez et al., 2008), our data indicate that overexpression of TRX-2 is ineffective in protecting hippocampal synapses against the adverse effects of a HCD. However, because we measured only one marker of oxidative stress in brain tissue samples,

it is possible that other markers of oxidative stress might be affected by the HCD and TRX-2 levels. Nevertheless, it is important to consider that a HCD can impair hippocampal LTP and learning by a mechanism other than increased oxidative stress. Previous studies suggest several such mechanisms for HCD-induced cognitive impairment including cellular insulin resistance (Stranahan et al., 2008a; Porter et al., 2010) and reduced neurotrophic factor levels (Molteni et al., 2002; Reichelt et al., 2015).

Learning and memory was impaired, and anxiety-like behavior was increased, in wild type and TRX-2 transgenic mice that had been maintained on the HCD. The lack of effect of TRX-2 levels on learning and memory or anxiety suggests that, at least within the range of hippocampal TRX-2 levels of the mice in the different groups in our study, TRX-2 does not modify the effects of a HCD on hippocampus-dependent behaviors. The roles of mitochondrial ROS in neuroplasticity are complex. Evidence suggests that superoxide and hydrogen peroxide can activate signaling pathways that regulate hippocampal neuroplasticity including those involving protein kinase C and MAP kinases (Knapp and Klann, 2002; Kamsler and Segal, 2003; Ma et al., 2011; Hou et al., 2013). Scavenging of mitochondrial ROS impairs hippocampal LTP suggesting critical roles for those ROS in synaptic plasticity (see Serrano and Klann, 2004). On the other hand, excessive production and/or reduced enzymatic removal of ROS can render neurons vulnerable to dysfunction and degeneration in experimental models of neurodegenerative disorders including Alzheimer's disease (Keller et al., 1998; Clausen et al., 2012). TRX-1 can protect neurons against insults relevant to ischemic stroke (Tian et al., 2014) and Parkinson's disease (Arodin et al., 2014a). It was reported that overexpression of TRF-2 can protect retinal ganglion neurons against oxidative stress and in a glaucoma model (Caprioli et al., 2009). However, levels of TRX1 are significantly increased in the brains of patients with mild cognitive impairment and Alzheimer's disease (Arodin et al., 2014b), suggesting either a contribution of TRX1 to the disease process or an adaptive response to neuronal damage. It remains to be determined whether hippocampal neurons of TRX-2 transgenic mice are more resistant to degeneration in experimental models of neurodegenerative conditions.

Finally, when taken together with the results of recent studies in animal models and human subjects, our findings suggest that dietary interventions aimed at reducing energy intake may improve hippocampal plasticity and cognitive function, particularly in overweight subjects. Whereas HCDs impair hippocampal function, caloric restriction and intermittent fasting can enhance hippocampal plasticity. For example, alternate day fasting increased hippocampal neurogenesis in mice (Lee et al., 2002) and protected rats against seizure-induced hippocampal neuron degeneration and associated cognitive deficits (Bruce-Keller et al., 1999). Rats that had been maintained on a reduced calorie diet exhibited reduced vulnerability to neurotoxin-induced hippocampus-dependent memory deficits (Qiu et al., 2012), and caloric restriction increased dendritic spine density of hippocampal dentate granule neurons in both normal mice and obese mice (Stranahan et al., 2009). Energy restriction may enhance cognitive function, in part, by modifying glutamate receptor subunit composition (Fontan-Lozano et al., 2007) and by enhancing neurotrophic factor signaling (Stranahan et al., 2009). We found that overexpression of TRX-2 did not ameliorate HCD-induced hippocampal synaptic plasticity and cognitive deficits. In contrast to a change in a single antioxidant protein such at TRX-2, energy restriction engages multiple adaptive

signaling pathways that together enhance synaptic plasticity and neuronal resistance to dysfunction and degeneration. Indeed, accumulating evidence suggests that antioxidant-based therapeutic approaches to neurological disorders may be untenable because they can paradoxically inhibit endogenous ROS-mediated signaling and adaptive cellular stress response pathways (Mattson, 2015).

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References

Arnold SE, Lucki I, Brookshire BR, Carlson GC, Browne CA, Kazi H, Bang S, Choi BR, Chen Y, McMullen MF, Kim SF. High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. Neurobiol Dis. 2014; 67:79–87. [PubMed: 24686304]

Arodin L, Miranda-Vizuete A, Swoboda P, Fernandes AP. Protective effects of the thioredoxin and glutaredoxin systems in dopamine-induced cell death. Free Radic Biol Med. 2014a; 73:328–336. [PubMed: 24863694]

Arodin L, Lamparter H, Karlsson H, Nennesmo I, Björnstedt M, Schröder J, Fernandes AP. Alteration of thioredoxin and glutaredoxin in the progression of Alzheimer's disease. J Alzheimers Dis. 2014b; 39:787–97. [PubMed: 24270206]

Bayer-Carter JL, Green PS, Montine TJ, VanFossen B, Baker LD, Watson GS, Bonner LM, Callaghan M, Leverenz JB, Walter BK, Tsai E, Plymate SR, Postupna N, Wilkinson CW, Zhang J, Lampe J, Kahn SE, Craft S. Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitiveimpairment. Arch Neurol. 2011; 68:743–752. [PubMed: 21670398]

Bonnard C, Durand A, Peyrol S, Chanseaume E, Chauvin MA, Morio B, Vidal H, Rieusset J. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J Clin Invest. 2008; 118:789–800. [PubMed: 18188455]

Caprioli J, Munemasa Y, Kwong JM, Piri N. Overexpression of thioredoxins 1 and 2 increases retinal ganglion cell survival after pharmacologically induced oxidative stress, optic nerve transection, and in experimental glaucoma. Trans Am Ophthalmol Soc. 2009; 107:161–165. [PubMed: 20126492]

Clausen A, Xu X, Bi X, Baudry M. Effects of the superoxide dismutase/catalase mimetic EUK-207 in a mouse model of Alzheimer's disease: protection against and interruption of progression of amyloid and tau pathology and cognitive decline. J Alzheimers Dis. 2012; 30:183–208. [PubMed: 22406441]

De Felice FG, Ferreira ST. Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease. Diabetes. 2014; 63:2262–2272. [PubMed: 24931033]

Du H, Guo L, Yan SS. Synaptic mitochondrial pathology in Alzheimer's disease. Antioxid Redox Signal. 2012; 16:1467–1475. [PubMed: 21942330]

Duchen MR. Mitochondria and Ca(2+)in cell physiology and pathophysiology. Cell Calcium. 2000; 28:339–348. [PubMed: 11115373]

Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB. Obesity, diabetes and cognitive deficit: The Framingham Heart Study. Neurobiol Aging. 2005; 26(Suppl 1):11–16. [PubMed: 16223549]

Farr SA1, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, Banks WA, Morley JE. Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology. 2008; 149:2628–2636. [PubMed: 18276751]

Finger BC, Dinan TG, Cryan JF. Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. Psychopharmacology (Berl). 2010; 210:559–568. [PubMed: 20422404]

Fontán-Lozano A, Sáez-Cassanelli JL, Inda MC, de los Santos-Arteaga M, Sierra-Domínguez SA, López-Lluch G, Delgado-García JM, Carrión AM. Caloric restriction increases learning

consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. J Neurosci. 2007; 27:10185–10195. [PubMed: 17881524]

Freeman LR, Haley-Zitlin V, Rosenberger DS, Granholm AC. Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms. Nutr Neurosci. 2014; 17:241–251. [PubMed: 24192577]

Grillo CA, Piroli GG, Evans AN, Macht VA, Wilson SP, Scott KA, Sakai RR, Mott DD, Reagan LP. Obesity/hyperleptinemic phenotype adversely affects hippocampal plasticity: effects of dietary restriction. Physiol Behav. 2011; 104:235–241. [PubMed: 21036186]

Hou Y, Mattson MP, Cheng A. Permeability transition pore-mediated mitochondrial superoxide flashes regulate cortical neural progenitor differentiation. PLoS One. 2013; 8(10):e76721. [PubMed: 24116142]

Hryhorczuk C, Sharma S, Fulton SE. Metabolic disturbances connecting obesity and depression. Front Neurosci. 2013; 7:177. [PubMed: 24109426]

Hsu TM, Konanur VR, Taing L, Usui R, Kayser BD, Goran MI, Kanoski SE. Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. Hippocampus. 2015; 25:227–239. [PubMed: 25242636]

Huang Q, Zhou HJ, Zhang H, Huang Y, Hinojosa-Kirschenbaum F, Fan P, Yao L, Belardinelli L, Tellides G, Giordano FJ, Budas GR, Min W. Thioredoxin-2 inhibits mitochondrial reactive oxygen species generation and apoptosis stress kinase-1 activity to maintain cardiac function. Circulation. 2015; 131:1082–1097. [PubMed: 25628390]

Hwang LL, Wang CH, Li TL, Chang SD, Lin LC, Chen CP, Chen CT, Liang KC, Ho IK, Yang WS, Chiou LC. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. Obesity (Silver Spring). 2010; 18:463–469. [PubMed: 19730425]

Kamsler A, Segal M. Hydrogen peroxide modulation of synaptic plasticity. J Neurosci. 2003; 23:269–276. [PubMed: 12514224]

Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav. 2011; 103:59–68. [PubMed: 21167850]

Karimi SA1, Salehi I, Komaki A, Sarihi A, Zarei M, Shahidi S. Effect of high-fat diet and antioxidants on hippocampal long-term potentiation in rats: an in vivo study. Brain Res. 2013; 1539:1–6. [PubMed: 24095795]

Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen HC, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J Neurosci. 1998; 18:687–697. [PubMed: 9425011]

Knapp LT, Klann E. Potentiation of hippocampal synaptic transmission by superoxide requires the oxidative activation of protein kinase C. J Neurosci. 2002; 22:674–683. [PubMed: 11826097]

Lowes DA, Galley HF. Mitochondrial protection by the thioredoxin-2 and glutathione systems in an in vitro endothelial model of sepsis. Biochem J. 2011; 436:123–132. [PubMed: 21355852]

Lu J, Holmgren A. Thioredoxin system in cell death progression. Antioxid Redox Signal. 2012; 17:1738–1747. [PubMed: 22530689]

Ma T, Hoeffer CA, Wong H, Massaad CA, Zhou P, Iadecola C, Murphy MP, Pautler RG, Klann E. Amyloid β-induced impairments in hippocampal synaptic plasticity are rescued by decreasing mitochondrial superoxide. J Neurosci. 2011; 31:5589–5595. [PubMed: 21490199]

Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, Ota T, Yokoyama M, Honda M, Miyamoto K, Kaneko S. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. Metabolism. 2008; 57:1071–1077. [PubMed: 18640384]

Mattson MP. Pathways towards and away from Alzheimer's disease. Nature. 2004; 430:631–639. [PubMed: 15295589]

Mattson MP, Gleichmann M, Cheng A. Mitochondria in neuroplasticity and neurological disorders. Neuron. 2008; 60:748–766. [PubMed: 19081372]

Mattson MP. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. Cell Metab. 2012; 16:706–722. [PubMed: 23168220]

Mattson MP. WHAT DOESN'T KILL YOU…. Scientific American. 2015 Jul; 313(1):40–5. [PubMed: 26204714]

Molteni R, Barnard RJ, Ying Z, Roberts CK, Gómez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience. 2002; 112:803–14. [PubMed: 12088740]

Morrison CD, Pistell PJ, Ingram DK, Johnson WD, Liu Y, Fernandez-Kim SO, White CL, Purpera MN, Uranga RM, Bruce-Keller AJ, Keller JN. High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling. J Neurochem. 2010; 114:1581–1589. [PubMed: 20557430]

Morrow JD, Roberts LJ 2nd. Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. Methods Enzymol. 1994; 233:163–174. [PubMed: 8015454]

Nicoll RA, Roche KW. Long-term potentiation: peeling the onion. Neuropharmacology. 2013; 74:18–22. [PubMed: 23439383]

Nonn L, Williams RR, Erickson RP, Powis G. The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. Mol Cell Biol. 2003; 23:916–922. [PubMed: 12529397]

Pérez VI, Lew CM, Cortez LA, Webb CR, Rodriguez M, Liu Y, Qi W, Li Y, Chaudhuri A, Van Remmen H, Richardson A, Ikeno Y. Thioredoxin 2 haploinsufficiency in mice results in impaired mitochondrial function and increased oxidative stress. Free Radic Biol Med. 2008; 44:882–892. [PubMed: 18164269]

Pivovarova NB, Andrews SB. Calcium-dependent mitochondrial function and dysfunction in neurons. FEBS J. 2010; 277:3622–3636. [PubMed: 20659161]

Porter DW, Kerr BD, Flatt PR, Holscher C, Gault VA. Four weeks administration of Liraglutide improves memory and learning as well as glycaemic control in mice with high fat dietary-induced obesity and insulin resistance. Diabetes Obes Metab. 2010; 12:891–9. [PubMed: 20920042]

Qiu G, Spangler EL, Wan R, Miller M, Mattson MP, So KF, de Cabo R, Zou S, Ingram DK. Neuroprotection provided by dietary restriction in rats is further enhanced by reducing glucocortocoids. Neurobiol Aging. 2012; 33:2398–2410. [PubMed: 22226488]

Reichelt AC, Maniam J, Westbrook RF, Morris MJ. Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelinexpression. Brain Behav Immun. 2015; 43:68–75. [PubMed: 25043993]

Ruggiero C, Ehrenshaft M, Cleland E, Stadler K. High-fat diet induces an initial adaptation of mitochondrial bioenergetics in the kidney despite evident oxidative stress and mitochondrial ROS production. Am J Physiol Endocrinol Metab. 2011; 300:E1047–1058. [PubMed: 21386058]

Serrano F, Klann E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Res Rev. 2004; 3:431–443. [PubMed: 15541710]

Sharma S, Zhuang Y, Gomez-Pinilla F. High-fat diet transition reduces brain DHA levels associated with altered brain plasticity and behaviour. Sci Rep. 2012; 2:431. [PubMed: 22666534]

Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. Int J Obes (Lond). 2013; 37:382–389. [PubMed: 22508336]

Sivanathan S, Thavartnam K, Arif S, Elegino T, McGowan PO. Chronic high fat feeding increases anxiety-like behaviour and reduces transcript abundance of glucocorticoid signalling genes in the hippocampus of female rats. Behav Brain Res. 2015; 286:265–270. [PubMed: 25721737]

Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus. 2008a; 18:1085–1088. [PubMed: 18651634]

Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat Neurosci. 2008b; 11:309–317. [PubMed: 18278039]

Stranahan AM, Lee K, Martin B, Maudsley S, Golden E, Cutler RG, Mattson MP. Voluntary exercise and caloric restriction enhance hippocampal dendritic spine density and BDNF levels in diabetic mice. Hippocampus. 2009; 19:951–961. [PubMed: 19280661]

Stranahan AM, Cutler RG, Button C, Telljohann R, Mattson MP. Diet-induced elevations in serum cholesterol are associated with alterations in hippocampal lipid metabolism and increased oxidative stress. J Neurochem. 2011; 118:611–615. [PubMed: 21682722]

Sultana R, Butterfield DA. Oxidatively modified, mitochondria-relevant brain proteins in subjects with Alzheimer disease and mild cognitive impairment. J Bioenerg Biomembr. 2009; 41:441–446. [PubMed: 19777328]

Tanaka T, Hosoi F, Yamaguchi-Iwai Y, Nakamura H, Masutani H, Ueda S, Nishiyama A, Takeda S, Wada H, Spyrou G, Yodoi J. Thioredoxin-2 (TRX-2) is an essential gene regulating mitochondria-dependent apoptosis. EMBO J. 2002; 21:1695–1703. [PubMed: 11927553]

Tian L, Nie H, Zhang Y, Chen Y, Peng Z, Cai M, Wei H, Qin P, Dong H, Xiong L. Recombinant human thioredoxin-1 promotes neurogenesis and facilitates cognitive recovery following cerebral ischemia in mice. Neuropharmacology. 2014; 77:453–64. [PubMed: 24212059]

Tucsek Z, Toth P, Sosnowska D, Gautam T, Mitschelen M, Koller A, Szalai G, Sonntag WE, Ungvari Z, Csiszar A. Obesity in aging exacerbates blood-brain barrier disruption, neuroinflammation, and oxidativestress in the mouse hippocampus: effects on expression of genes involved in beta-amyloid generation and Alzheimer's disease. J Gerontol A Biol Sci Med Sci. 2014; 69:1212–1226. [PubMed: 24269929]

Wang Y, Greig NH, Yu QS, Mattson MP. Presenilin-1 mutation impairs cholinergic modulation of synaptic plasticity and suppresses NMDA currents in hippocampus slices. Neurobiol Aging. 2009; 30:1061–1068. [PubMed: 18068871]

Warneke W, Klaus S, Fink H, Langley-Evans SC, Voigt JP. The impact of cafeteria diet feeding on physiology and anxiety-related behaviour in male and female Sprague-Dawley rats of different ages. Pharmacol Biochem Behav. 2014; 116:45–54. [PubMed: 24269545]

White CL, Pistell PJ, Purpera MN, Gupta S, Fernandez-Kim SO, Hise TL, Keller JN, Ingram DK, Morrison CD, Bruce-Keller AJ. Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: contributions of maternal diet. Neurobiol Dis. 2009; 35:3–13. [PubMed: 19374947]

Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJ, Vos T. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet. 2013; 382:1575–1586. [PubMed: 23993280]

Wu A, Ying Z, Gomez-Pinilla F. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. Eur J Neurosci. 2004; 19:1699–1707. [PubMed: 15078544]

Yau PL, Castro MG, Tagani A, Tsui WH, Convit A. Obesity and metabolic syndrome and functional and structural brain impairments in adolescence. Pediatrics. 2012; 130:e856–864. [PubMed: 22945407]

Zhang J, Wang Y, Chi Z, Keuss MJ, Pai YM, Kang HC, Shin JH, Bugayenko A, Wang H, Xiong Y, Pletnikov MV, Mattson MP, Dawson TM, Dawson VL. The AAA+ ATPase Thorase regulates AMPA receptor-dependent synaptic plasticity and behavior. Cell. 2011; 145:284–299. [PubMed: 21496646]

Highlights

- A high calorie diet impairs synaptic plasticity and learning in mice
- Mice overexpressing TRX-2 exhibit resistance to oxidative stress
- TRX-2 fails to protect hippocampal synapses against a high calorie diet

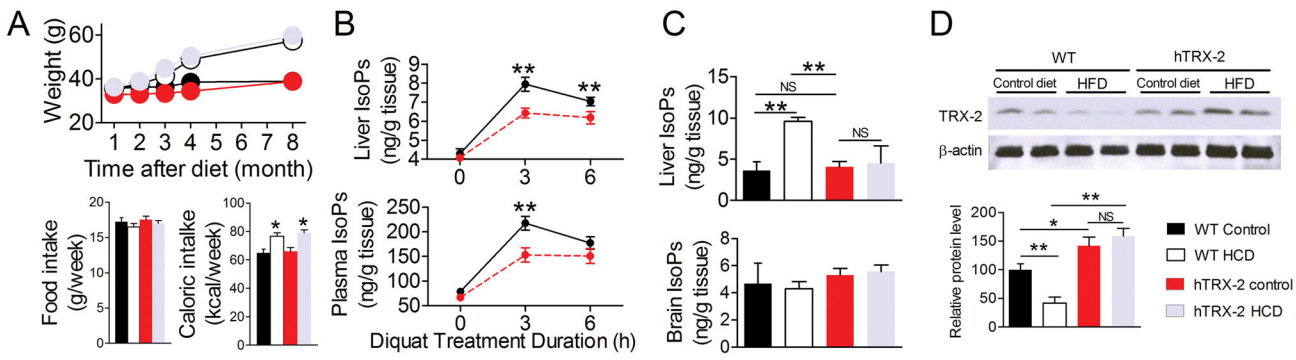

Figure 1.

Overexpression of hTRX-2 protects peripheral tissues against oxidative stress caused by a high calorie diet, whereas the hippocampus is relatively resistant to HCD-induced oxidative stress. **A.** Body weights, food intake and caloric intake of wild type and TRX-2 transgenic mice maintained on either a control diet or a HCD (10 mice per group). From the third month of diets, the weight gain of WT or TRX-2 mice under HCD were both dramatic higher compared with those under control diet separately ($p<0.01$). No significant difference were found between WT and TRX-2 wither HCD or control diet ($p>0.05$). No significant difference of food intake were found betwwen all groups ($p>0.05$). **B.** Plasma and liver isoprostane levels in wild type and TRX-2 transgenic mice treated with diquat (50 mg/kg i.p.) for the indicated times (5 mice per group). **C.** Liver (upper) and hippocampal (lower) isoprostane levels in tissue samples from wild type and hTRX-2 transgenic mice that had been maintained for 8 months on either a control diet or a HCD (3 mice per group). **D.** Immunoblot analysis of TRX-2 protein levels in hippocampal tissue of mice in the indicated groups (6 mice per group). Data represent the mean ± s.e.m.: *$p<0.05$, **$p<0.01$; NS, not significant.

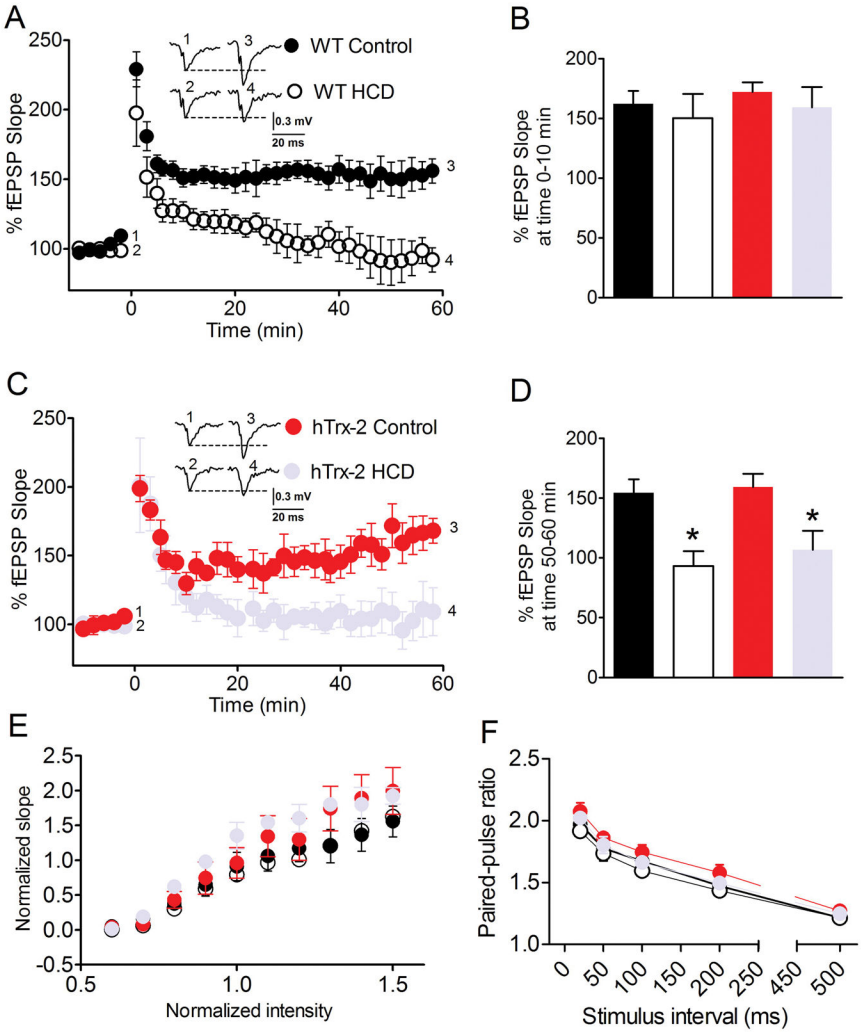

Figure 2.

Mice maintained on a high calorie diet exhibit impaired long-term potentiation at hippocampal CA1 synapses, and this deficit is unaffected by TRX-2 overexpression. **A – D.** Results of field EPSP recordings at CA1 synapses of hippocampal slices from wild type or hTRX-2 transgenic mice that had been maintained for 8 months on a control diet or a HCD. Panels A and C show the field recordings, and panels B and D show average EPSP slope measured at either 0–10 minutes or 50–60 minutes post-tetanus. **E and F.** Input-output curves (E) and paired-pulse ratio data (F) from recordings from hippocampal slices from mice in the indicated groups. Values are the mean and SEM of data from recordings from 8 slices from 4 mice/group. *$p<0.01$ compared to the value for mice in the control diet groups.

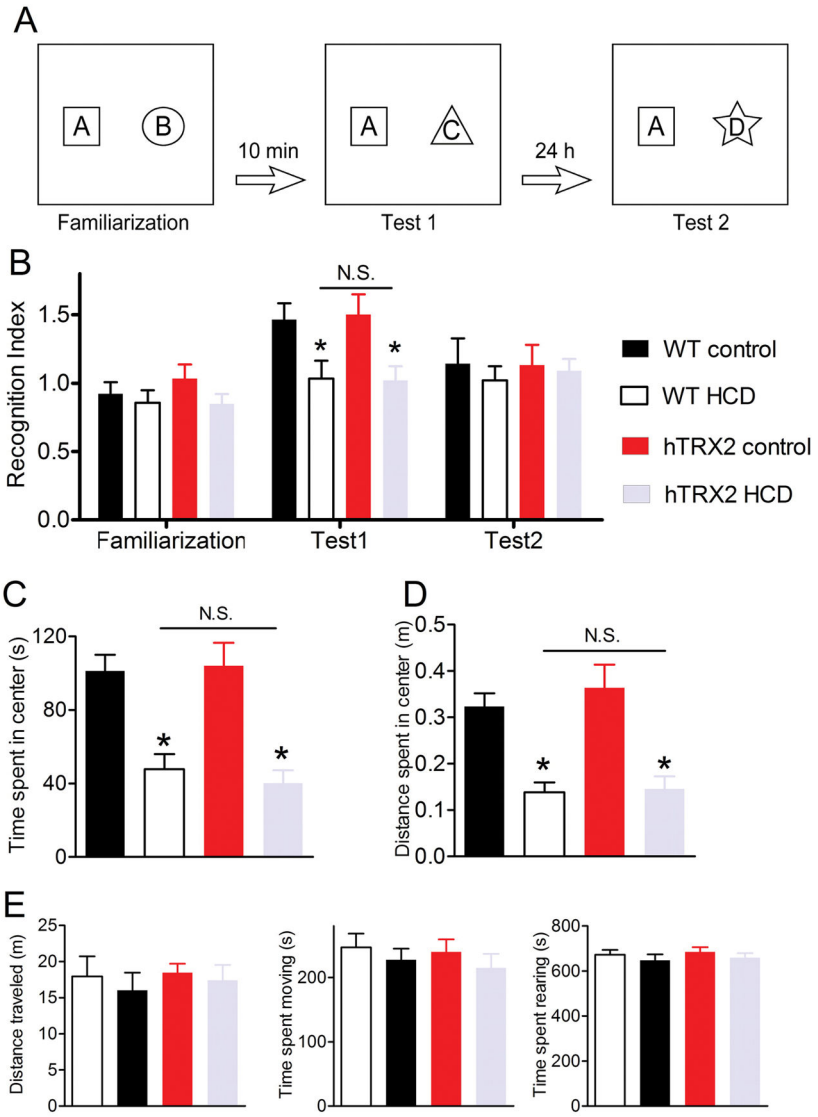

Figure 3.

Mice maintained on a high calorie diet exhibit a deficit in hippocampus-dependent learning and memory, and heightened anxiety, and these behavioral alterations are unaffected by hTRX-2 overexpression. Behavioral performance in novel object recognition and open field tests in wild type and hTRX-2 transgenic mice that had been maintained for 8 months on either a control diet or a HCD. **A.** Illustration showing the experimental design of the novel object recognition test. **B.** The recognition index, represents the ratio of the time period the mice spent exploring the novel object (objects 'B', 'C' or 'D' in panel A) compared to the time spent exploring the familiar object ('A') in panel A. **C and D.** Results of open field test showing time spent in the center zone (C) and distance traveled in the center zone (D). **E.** Results of open field test showing total distance traveled, total time spent moving and total time spent rearing. Values are the mean and SEM of data from 8–10 mice/group. *p<0.05; NS, not significant.