Comforting food: the mechanisms underlying the effect of high fat diet on restoring social deficits following social isolation

1. **Scientific background**

Loneliness is increasingly recognized as a serious threat to mental health 1,2. Recently and due to the COVID19 pandemic social isolation emerged as a major risk factor for psychiatric and emotional disorders development. The quarantine caused by COVID-19 has increased domestic violence, fear of people losing their jobs, reduced physical activity, altered sleep, and increased anxiety [ Reviewed in 3]. **While research focuses on adults, the effects of loneliness in children and adolescents is under-studied.**

Social isolation is one of the most prominent stressors in animal models 4. One of the widely used models in rodents is post-weaning isolation rearing, which involves isolation of the animals, starting at a very early stage (postnatal day (PND) 20–28) without handling and for an extended period of several weeks or months5. Previous studies reported that juvenile social isolation for 2 weeks from PND 21 to 35, was associated with decreased sociability in adulthood and hypomyelination in deep layers of the medial prefrontal cortex (mPFC), a critical node for social behaviors6,7. Re-socialization with socially isolated mice did neither rescue the impaired sociability nor the hypomyelination in the mPFC6 (but see 7), indicating that social interactions between previously socially isolated mice are not sufficient to restore these deficits. Similarly, post-weaning isolation induced an increase in inhibitory synaptic activity and decreased intrinsic excitability in pyramidal cells in the mPFC in the adult mice that were neither restored by regrouping nor by chemogenetic or optogenetic manipulations that resulted only in a transient reversing effect 8.

The post-weaning period is a critical developmental window for the maturation of the mPFC, that is required for lifelong emotional and social memory9, suggesting that environmental factors like exposure to stressful adversities during juvenility can affect the trajectory not only of the mPFC but also of its projections to other parts of the brain. These findings suggest that developing avenues to reverse the deficits caused by social isolation/ distancing/ loneliness in children and adolescents is critical as it is a period that sets the stage for lifelong mental health 10,11.

Studies support the hypothesis that an “unhealthy” but palatable diet decreases the impact of stress exposure. Indeed, an increase in consumption of calorie-rich palatable foods was found to engage certain coping mechanisms to reduce stress in humans and in rodents 12–14. It was previously shown that long-term exposure to high-fat diet (HFD) selectively protects against some of the behavioral sequelae of chronic unpredictable social stressors 15 and protects offspring’s from the consequences of maternal separation stress 16. Studies mainly focus on addressing the long-term consequences of social isolation or exposure to HFD when animals reach adulthood; however, the effects of these manipulations are not frequently examined in young animal. In our work we focus on the immediate effects of exposure to stressors (acute stress) on behavior and plasticity in juvenile animals17–22. In our preliminary studies (Figures 1-3), social isolation, the effects of HFD or their combination, were applied to rats one week starting from PND21. Social memory, as assessed by short-term and long-term social recognition memory (habituation and dishabituation and social recognition memory respectively), and mPFC synaptic plasticity, as assessed through NMDA-dependent long-term potentiation (LTP) were equally impaired by social isolation or HFD intake during the juvenile period as we previously shown for HFD18,23–25 (Figures 1-3). Interestingly, although HFD have been shown to impair social memory under normal conditions, when it is provided during isolation it is protective against the negative effects of isolation as placing the isolated animals on HFD rescued the deficits in social memory and LTP (Figure 1-3). Notably, this behavioral and electrophysiological recovery persisted a month later, a time point at which social isolation still has an impairing effect on social memory and LTP (Figure 4). These findings correspond with a previous study reporting that chronic exposure to HFD after isolation improved isolation-induced deficits in object recognition memory and decreased BDNF levels in mPFC26. Another study showed that mice previously fed either high fat or high sucrose diets exhibited increased rearing behavior in the elevated zero maze 30 days post stress and diet exposure. The authors concluded that short-term diet administration can initiate a long-term increase in risk-assessment27. These suggest that palatable diet (high fat or sugar) under stress conditions can affect the stress response.

We have recently showed that social isolation of adult animals for one week impaired social memory and decreased BDNF levels in the medial amygdala, another important node of social behavior, yet both effects were reversed by regrouping of the isolated animals 28. Similarly, an increase in BDNF was found in the hippocampus following 72 hrs of HFD consumption in the adult animal that also resulted in improved spatial memory 29. All together, these may suggest that HFD/ isolation or the combination of both may have affected the reprogramming of the mPFC neural networks and resulted in long-term effects (and maybe of other circuits like the amygdala and the hippocampus).

In search of molecular underpinning of social isolation, a growing body of evidence indicated that social isolation in rodents affects the expression of non-coding RNAs like miRNAs30–32. miRNAs are a class of ~22 nucleotide short non-coding RNAs that play key roles in fundamental cellular processes, including how cells respond to various stress conditions 33–36. In the brain, miRNAs impact cellular and subcellular functions and modify cognitive performance 37,38 as was also demonstrated in HFD-induced deficits 39. A recent study addressed the effects of social isolation in middle aged and aged mice and pointed out specific [miRNA](https://www.sciencedirect.com/topics/chemistry/microrna) signatures (miR-140-5p and miR-181a-5p) in the mPFC. These miRNA alterations were reversible and were back to control levels following enrichment 40. Prolonged isolation of postnatal rats resulted in differential miRNAs expression in brain regions involved in anxiety responses 31. For example, some miRNAs were differentially regulated in both socially-isolated males and females rodents, with the majority being downregulated (reviewed in 3). Similarly, studies have characterized the contribution of miRNAs expression in the PFC to PFC-dependent tasks 37,41. Furthermore, studies suggest that miRNA-135a and miRNA-16 are important candidates for understanding the mechanisms by which stressful early-life experiences increase post-traumatic disorder (PTSD) vulnerability42. Our preliminary study with Prof. Irit Akirav on the effects of early life stress on the expression of miRNA-16 and the effects of antagonizing it (See preliminary data) showed that indeed antagonizing miRNA reduced its expression in the mPFC (Figure 5A). We further showed that, ELS down-regulated the expression of miR-16 and chronic treatment with URB normalized this effect (Figure 5B). Thus, it is likely that miRNAs and their target genes are key components of the mechanism that underlies the deficits caused by each of the factors (HFD, isolation) and the rescue of deficits that is caused by HFD under social isolation.

Our results may fit in the framework of resilience; changes induced by each condition separately are rescued when the two conditions are presented simultaneously. For example, we previously showed that a single exposure to the elevated platform stress impairs prefrontal LTP, whereas two exposures to this stressor result in intact mPFC LTP 43–45. Previous studies, identified miR-218 as a susceptibility *versus* resilience molecular switch between in stress-related disorders by a mechanism that involves the DCC Netrin-1 guidance cue receptor 46,47. This Netrin-1 participates in the organization of neuronal circuitry across the lifespan and plays a critical role in the maturation of PFC during adolescence48,49. These findings may position miRNA and their target genes further indicate their possible central role in the deficits caused by social isolation or HFD intake in the juvenile animal. The **central hypothesis** of this proposal is that ***HFD under social isolation causes significant, age- and brain region-specific reprogramming of miRNAs and gene expression that enduringly affect neuronal plasticity and memory.***

1. **Objectives and significance of the research**

In the current application, we endeavor to explore into depth the mechanisms by which social isolation and HFD lead to deficits in social memory and LTP and how HFD under social isolation rescued social deficits induced by each of the conditions separately. We propose to examine the cellular, molecular and behavioral modifications at circuit level (mPFC, the hippocampus and the amygdala) to understand the signature of each of these factors, and their interaction, on the reprogramming changes that occur at each of these components of the circuit.

We will focus on miRNA mapping. Since miRNAs have the potential to titrate expression levels of multiple protein targets, creating complex miRNA-mRNA interaction patterns, we will identify these targets with the ultimate goal to enhance or inhibit the targeted miRNA (by agomirs and antagomirs, respectively) to establish a mechanistic link.

**To address these objectives, we have the following aims:**

1. **Behavioral, cellular and electrophysiological profiling of the consequences of social isolation, HFD and the combination of both in the mPFC-amygdala-CA1 neuronal network.**
2. **Identify putative miRNA-regulated genes and pathways underling the phenotypes of HFD, Social isolation and the combination of Social Isolation + HFD in the mPFC by conducting multi-omics analysis.**
3. **To explore whether inhibiting or activating specific candidate miRNAs can reverse cognitive and emotional dysfunction related to social isolation and HFD**

Changes in miRNA following these manipulations will be examined at the circuit level and thus providing information how the network changes. As stress and HFD result in different effects in the hippocampus and the PFC as compared to the amygdala in an age-dependent manner17,50–53, the comparisons proposed here will allow us to examine whether the modifications are differently regulated in the three brain structures. We will further address the temporal dynamics of these signatures and biomarkers by looking at short or long-term consequences.

To the best of our knowledge, this proposal is the first to address these questions while addressing the effects of behavioral, cellular, and molecular effects of social isolation, HFD and the combination of the two at circuit level while covering two developmental phases (juvenile and adult periods).

**Significance:**

Social isolation, during childhood in particular, is detrimental to adult brain function and behavior across mammalian species 6,54. While the majority of research addresses extended periods of isolation and monitor the effects in the adult animals, here we propose a different approach that uses a short and acute isolation period while assessing its immediate in the juvenile animal but also its long-term effects. Dissecting the circuit that mediates social behavior and the effects of isolation on social behavior as well as how different types of diets that are becoming popular and accessible might mitigate the behavioral effects of juvenile stress may be very relevant in light of increase in social distancing. It is important to note that our acute exposure to HFD was not associated with any metabolic disturbances or overweight 23–25,55, suggesting that palatable food can be an avenue to explore without the negative effects of obesity 56. We are aware that recommending on fatty diet to resolve social disturbances in children might be controversial. However, our proposal lies on a strong basis of published and preliminary data and in line with the literature, and thus it may open an avenue to characterize the micronutrient that may improve social disturbances especially in children without the harmful aspect of the diet. As such, this might constitute a tailored-made personalized diet treatment under control of professionals for buffering against the detrimental effects of social isolation.

**Aim 1: Behavioral, cellular and electrophysiological profiling of the consequences of social isolation, HFD or other diets (high fat or high sugar) and the combination of both in the mPFC -BLA-CA1 network**

**Rationale:** We previously reported devastating effects of 1 week of HFD in juvenile animals on social memory and neuronal plasticity in the CA1 and mPFC 23–25,55.In the adult animals, HFD did not affect social memory 23,55and induced mixed effect on neuronal plasticity, with adult HFD enhancing CA1 LTP 21,29 but impairing LTP in the mPFC46, showing region-dependent changes. Social isolation in adults resulted in deficits in both social memory28 and mPFC-LTP (preliminary results; Figure 5) showing that social isolation at adulthood is detrimental. Exposure to HFD in isolated adult animals reversed completely social memory impairments but only partially mPFC LTP deficits, suggesting that this combination is more powerful in juveniles. In contrast to this robust effect of isolation+HFD, research reported that regrouping or chemogenetic manipulations of mPFC projections had transient effect only 28,57. Our experimental setting proposed here provides changes in both directions (impairment to reversal), has different effects in adults and juveniles, yields different effects in different brain regions, and is robust with enduring long-term effects. We believe that this model is ideal for understanding the effects of acute social isolation on the juvenile or adult brain in both males and females.

**Aim1a: Behavioral profiling the effects of social isolation, HFD and the combination of both in juvenile and adult males and females.**

We will test animals either immediately or 1 month after the 1 week of isolation, HFD or both on social memory but also on non-social memory tasks, like object location memory (OLM), and emotional behavior (anxiety-like behaviors) to address whether isolation induces specific social memory deficits or more general cognitive and emotional impairments. As exposure to HFD after the termination of isolation resulted in a slight improvement 26, this Aim will also address temporal contingency by addressing whether 1 week of HFD immediately after the termination of isolation will result in similar reversal. This will show whether there is a critical window for the combination of both conditions.

**Aim 1b: Dissecting the brain regions and circuits activated following social isolation, HFD and the combination of both in juvenile and adult males and females.**

Here, we propose to examine patterns of c-Fos expression following the different conditions to identify brain regions with potentially differential activity that might contribute to the effect of isolation, HFD and both on social memory. We will focus on the c-Fos activation of the mPFC, CA1 and the amygdala (medial and basolateral nuclei) upon social exposure in the different conditions (Age, Diet, and Housing). We have preliminary data showing that following social recognition memory under standard control diet, there is a differential recruitment of the mPFC, in juvenile males as compared to adults. It will be thus interesting to examine whether under isolation, HFD or both there will be differences in the recruitment of the mPFC, CA1 and the amygdala in the two groups of age.

**Aim1c: Cellular signature to the isolation, HFD and both**

Our preliminary results of LTP were performed in the mPFC, we did not examine the effects of these manipulations in the CA1 or the basolateral amygdala (BLA). Here we will use high frequency -induced LTP to address deficits and rescue by the different conditions on the network. This classical method yielded in our and other labs exciting results regarding the differential effects of stress on plasticity in juveniles and adults17,19,22,43,44,53.

**Working hypotheses:** We expect that juvenile and adult animals under isolation and HFD will show restoration in SRM as well as OLM and anxiety. The rescue of memory deficits expected in Aim1a will be accompanied by normalization of LTP changes in the CA1 and the mPFC, but not in the BLA (see our previous publications17,18,53). The rescue may also be reflected in differential activation as monitored by c-Fos compared to HFD and isolation groups. We further predict that the rescue will be optimal when HFD+isolation are presented together (instead of successively), during the juvenile period.

**Research design and methods:** All experiments and protocols proposed in this proposal will be performed according to the regulations of the ethical committee at the University of Haifa for animal experimentation and welfare. We have experience in all protocols proposed here; behavioral testing as well as LTP are previously described 18,19,22–25,51, c-Fos immunostaining and the protocols are described in our published work 58,59. We will test [Age (Juveniles, Adults), Housing (Isolation, Group), Diet (HFD, CD)] at 7 or 30 days after manipulations. For behavior animals will be tested after the 7 or 30 days period, some will be taken to electrophysiology or will be euthanized 90 min after the last behavioral manipulation. We will test also naïve groups in both electrophysiology and c-Fos as additional controls. We will employ the three R policy to reduce the number of animals. For each region, we will quantify the number of c-fos+ neurons from both left and right hemispheres and the average within each animal.

**Expected results and pitfalls for Aim 1**: It is expected to detect differences in juveniles and adults and this will be observed by robust changes in juveniles compared to adults. If we find that social isolation also impairs cognitive tasks and that HFD under isolation can rescue cognitive deficits, this may indicate that isolation and HFD under isolation may have more generalized effects that are not only restricted to social behavior. If we find overlap in the c-Fos activation after HFD and isolation, this may indicate shared neural networks.

My lab has expertise in all the required protocols and thus we do not expect any technical challenge.

### Aim 2: The molecular basis of the effects of HFD, isolation and the combination of both

**Rationale:** A body of evidence relate the effect of social isolation, HFD and other stressors to miRNA expression profile in different brain regions, including the mPFC 39,41,46,48,60–62. Therefore, involvement of miRNA in the re-programing of mPFC gene expression following dual exposure to social isolation and HFD is highly requisitioned. Furthermore, as miRNA regulate protein expression (directly and also indirectly), concurrent examination of mPFC proteome is required. Indeed, studies have recorded changes in proteomic profiles in different brain regions following social isolation 60,63as did our own study of the medial amygdala28. We aim to obtain genome-wide analysis of both miRNA and proteomic profiles of the mPFC as affected by social isolation, HFD and their combination. Using integrated data from both miRNA and proteomic profiles, we will be able to identify uniquepatterns of miRNA expression and complementary protein and molecular pathways alteration of expression. Integration of the both datasets is key to gaining robust hypotheses for molecular mechanisms underlining deficiencies and their rescue.

**Working hypotheses:** Our preliminary data show that isolation and HFD, separately exert devastating effects on memory processes. The rescue by the combination of the two (isolation+HFD) suggests that molecular reorganization could mediate the recovery. MiRNAs are defined as important elements that may connect environmental factors with gene expression. We thus predict that different miRNAs will be expressed in the three conditions (isolation, diet and the combination) and different protein expression pathways will accompany these. We predict also differences in the pattern of expression in the three brain regions (mPFC, amygdala and hippocampus). In accordance with these assumptions on differential expression of miRNAs in different brain regions, a previous study reported that the central amygdala and the CA1 of the Hippocampus displayed distinct clusters of miRNAs following either acute or chronic stress35.

**Research design and methods:** In order to identifymolecular mechanisms involved in the deficiencies following either social isolation or HFD and the rescue in the combined treatment, integrated miRNA and proteomic analysis will be performed. Due to the immense costs of transcriptomic and proteomic analyses, we will focus here on the mPFC in juvenile and adult rats and 1 week after the treatment as we showed it has a robust effect. For each group, eight animals will be sampled after the termination of the 1-week exposure. We will extract total RNA including miRNA as well as proteins from the same sample. From the same animals, the hippocampus and amygdala will also be taken for further study at later stage. Total RNA and proteins will be extracted from the samples as described in our previous study28 . miRNA-seq library construction and sequencing will be done at the Technion Genome Center (Technion, Israel). Pre-processing as well as identification and quantification of miRNA expression will follow the workflow proposed by Yao et al. 64. Briefly, following quality control (adapter sequences were removed and read-quality filtration), high-quality reads will be mapped to several small RNA debases (including miRBase, piRBase and GtRNAdb) as well as to the latest NCBI versions of the *Rattus norvegicus* transcriptome and genome (GenBank assembly mRatBN7.2 under accession: GCA\_015227675.2). Novel miRNA discovery will be conducted using the miRDeep2 algorithm 65. Additionally we will assess putative mRNA targets of the identified miRNA based on current databases (*e*.*g*., miRDB 66 Label- free LC–MS/MS analysis and determination of proteomic profiles will be conducted at The Smoler Protein Research Center (Technion, Israel). The database for retrieval of the secondary mass spectrometry data will be Uniprot rat reference proteome and label-free Quantitation (LFQ) values will be assigned. The datasets obtained (miRNA x sample and protein x sample) will then be analyzed for differential expression and functional gene-set enrichment. First, each set will be analyzed individually, following our previous workflow28. Next, the two datasets will be integrated using graph convolutional networks, as implemented in *e*.*g*., MOGONET 67 a supervised multi-omics integrative analysis approach. This approach will allow the identification of unique biomarkers (both miRNA and proteins) specific to group and/or phenotype. Dr. Maya Lalzar head of the "University of Haifa Bioinformatics Services Unit" will conduct Bioinformatics analyses.

**Expected results and pitfalls for Aim 2**: We expect to provide insight into the molecular mechanisms underlining social memory impairment in the rat mPFC under two scenarios: social isolation and HFD and identify miRNA and proteins biomarkers. Importantly, we will identify the molecular components and pathways that confer resilience under condition of combined social isolation and HFD that occurs in juveniles but not in adults. At the conclusion of this aim, we will propose a list of miRNA (and possibly their putative mRNA targets) putatively governing gene expression remodeling under the different conditions. Still, bulk tissue analyses in brain research have been criticized for high-masking of effects, many time occurring only in a small subset of cells within the tissue. Therefore, our identification of molecular elements may be partial rather than comprehensive. Nevertheless, our previous experience shows that impact of social isolation on transcriptomic and proteomic profiles is readily accessible through bulk RNA and protein processing and analysis 28. Additionally, we may find that co-isolation of total RNA and total protein from the same sample may affect quality or quantity of the sample. In such case, we will conduct the extraction of the different components separately, using left and right hemispheres (counterbalanced).

**Aim 3: To explore whether inhibiting or activating specific candidate miRNAs can reverse cognitive and emotional dysfunction related to social isolation and HFD**

**Rationale:** After identifying the candidate miRNAs in the previous aim, here we will aim to establish a causal link by directly manipulating specific miRNA by infusing antagomirs/agomirs in the mPFC, and hippocampus or amygdala according to what was profiled in the previous aims.

The list of miRNA identified in aim 2, will be cross examined against a list of miRNA candidate miRNA compiled from the literature (specific miRNAs based on the literature on social isolation, obesity-HFD). We will rank the different miRNA based on all evidence available and select xxx for validation by reverse-transcription quantitative PCR (RT-qPCR).

For the set of RT-qPCR confirmed miRNAs, we will conduct first microinfusion in the mPFC of antagomirs/agomirs. For those miRNA with high rescue efficiency, for either social isolation, HFD or social isolation+HFD we will further examine expression of predicted protein targets (based on the findings in Aim 2) by Western blot analysis. Additionally, for the infusion-confirmed miRNAs, we will examine expression in the hippocampus and amygdala of male rats (sampled at the first stage of this aim).

**Working hypothesis**: Based on the predictions from the previous Aim2 and predictions according to the literature, we hypothesize that activating specific miRNAs that were differentially upregulated in the Isolation + HFD condition will rescue deficits in SRM and LTP. We further expect that these effects will persist 1 month later and that the effects of these manipulations are not only region specific (mPFC vs BLA) but also age-dependent with juvenile animals showing the most robust effect.

**Expected results and pitfalls: This Aim 3 attempts to address the mechanistic link of the most relevant miRNAs expressed in the rescue effect and how** mimicking and blocking these miRNA using agomirs and antagomirs **will affect the phenotype of the three groups. Thus, we expect that blocking** these miRNAs in the isolation+HFD condition will impair SRM and LTP and mimicking them will prevent the deficits caused by social isolation or HFD. As the mPFC and amygdala present opposing effects, we predict differential effects of activation and inhibition. **Regarding the long-term effect of miRNAs manipulations, we might address** how long the effects of the agomirs and antagomirs last. If the expression of the specific miRNAs following the microinjection does not last 1 month, we may consider examining at 1 or 2 weeks after the termination.

**Research design and methods:**

**Validation of miRNA expression by RT-qPCR**: We will target under the different conditions (social isolation/ no social isolation, juveniles/ adults, HFD/CD, 1 week/1month). Tissues from the mPFC, BLA, MeA and CA1 will be tested for the expression of miRNA. Briefly, samples from the punched area will be extracted bilaterally and RNA extraction followed by cDNA synthesis will be performed according to Zaidan et al., (see support letter). The expression of list of candidate miRNAs will be assessed using SYBR Green qRT-PCR amplification. Fold-change values will be calculated using the ddCt method 68 relative to the housekeeping genes RNU6 and RNU66.

**Western blot analysis: According to our previous work**

**General Pitfalls**: **Females:** Social and anxiety disorders are more evident in women than in men yet animal models focus mainly on adult males. We chose to focus here on males due to the enormous amount of work in this proposal and as we have mainly results on males. However, we do not exclude comparison with females and this is will be considered at least in Aim 1, and if differences will be evident then we will include females in Aim 2 and 3.

**HFD vs Western and high glucose diet**: The HFD that we characterize here may not be the type of western diet (mixed fat and sugar) that is increasingly consumed in young children and adolescents. We may consider comparison to other palatable diets. We focused on only one diet (HFD) due to the large amount of work required.

**Correlation with behavior**: Another potential challenge is that the behavioral and physiological assays would likely affect the brain transcriptome. Therefore, different animals that are exposed to the different conditions yet not being put through the battery of tests would have to be used for the RNAseq analyses. This might increase the variability and therefore might require more animals to be tested.

Analysis: It is possible that we will uncover multiple transcriptome changes caused by the different conditions and it would be difficult to pinpoint which ones are primarily responsible for the phenotype observed previously. In this case, additional refinements might be necessary. For example, we may increase number of animals. Even if all these refinements will be insufficient to pinpoint the primary transcriptome changes associated with the different conditions, the transcriptome data generated in this work will serve as the basis for subsequent studies in the very important area of relationship between nutrition and brain functions.

Technical challenge: My lab has wide expertise in behavior, pharmacology, electrophysiology, immunohistochemistry. Our exciting preliminary data requires stepping out of the comfort zone and we have guarantee technical support using miRNAs from the laboratory of Prof Irit Akirav with whom we have joint projects on the effects of early life stress on mPFC (preliminary data # XX). We further have technical support from the Unit of Bioinformatics at the Haifa University

**General methodology:**

Diets: [HFD: 20% derived from protein, 60% fat (mainly from lard), 20% carbohydrates (6.8% from sucrose); HFG: 17% protein, 40% fat (butter and corn oil), and 43% carbohydrates (mainly glucose);  CD: 16% protein, 4% fat, 80% carbohydrates (mainly starch)].



**Figure 1: Acute effect of HFD and social isolation for one week on** social recognition memory **in the juvenile animal.**

**A:** Schematic presentation of the social recognition memory test: Animals are habituated to the arena, and on the next day they are exposed to an object or to another animal placed in corrals for 1 hr. Twenty-four hrs later they are exposed to the familiar animal and to a novel one. Details in 23,28.

**B:** Animals at PND21 were weaned and divided into 4 groups: control diet and group housing (J-CD-Social), CD and isolation (J-CD-Isolation), High-fat diet and group housing (J-HFD-Social) and HFD and isolation (J-HFD-isolation). Immediately after the termination of the 7 days they were tested on social recognition memory test. ANOVA showed a significant interaction between housing and diet [F(1, 28)=79.5. P<0.0001]. Follow up analysis showed that while CD-isolation and HFD-isolation did not differ from each others [ns], they significantly differed from the other groups [p<0.001]. The CD-social and HFD-isolation groups showed intact social recognition memory.



**Figure 2: Acute effect of HFD and social isolation for one week on short-term recognition memory of social habituation and dishabituation** in **the juvenile animal.**

**A:** Schematic presentation of the short-term social recognition memory test: Animals are repeatedly presented with the same conspecific for 4 trials (F1 X 4), the trials are separated by 10 minutes interval. On the fifth trial, animals are presented with a novel conspecific. Details in 23,28.

**B:** Animals at PND21 were weaned and divided into 4 groups: control diet and group housing (J-CD-Social), CD and isolation (J-CD-Isolation), High-fat diet and group housing (J-HFD-Social) and HFD and isolation (J-HFD-isolation). Immediately after the termination of the 7 days, they were tested on social recognition memory test. ANOVA showed a significant interaction between housing and diet [F(1, 28)=79.5. P<0.0001]. Follow up analysis showed that while CD-isolation and HFD-isolation did not differ from each others [ns], they significantly differed from the other groups [p<0.001]. The CD-social and HFD-isolation groups showed intact social recognition memory.



Figure 3: The effects of HFD, social isolation and the combination of both on LTP recorded 60 min following the application of high-frequency stimulation (A) and the averaged LTP (B).

HFD and social isolation, separately resulted in impairments in High frequency-induced LTP in the mPFC. Potentiation was rescued by the combination of both social isolation and HFD.



**Figure 4:** **long-term effect of HFD and social isolation for one week at juvenility on social recognition memory (A) and LTP (B) tested at adulthood.**

Animals at PND21 were weaned and divided into 4 groups: control diet and group housing (J-CD-Social), CD and isolation (J-CD-Isolation), High-fat diet and group housing ( J-HFD-Social) and HFD and isolation (J-HFD-isolation). Immediately after the termination of the 7 days, they were placed back in group housing and CD. Animals were tested at PND 60. (A) ANOVA showed significant interaction between housing and diet [ F(1, 33)=5.9, P<0.001]. Follow up analysis showed that the J-CD-social was not different from the J-HFD-isolation and the two groups showed intact memory. The CD group that was isolated as well as the HFD group that was in group housing showed long-term impairments in social recognition memory.

(B) ANOVA showed significant interaction with diet and housing [ F(1, 24)=62.9, P<0.001). Follow up analysis showed social isolation+HFD showed intact LTP, similar to Social-CD animals. Interestingly, isolation-CD showed impaired LTP (100 ±3.6%) and was significantly different from the social-HFD group which showed attenuated levels of potentiation but the levels of potentiation were moderate (117.9±2.4%),

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**Figure 5:**

**A:** Rats were microinjected with antagomir-16 (anti-mir, 20 nm; Creative Biiogene) into the right ventricle and decapitated after 7 weeks. A significant decrease was observed in the expression of mir-16 in the PFC (n=5 in all groups) (\*, p<0.05).

B: The effects of early life stress (ELS) and chronic treatment with URB597 during late-adolescence on the expression of miR-16 in adult male rats in the mPFC. (\*, p<0.05; \*\*\*, p<0.001; #, p<0.05 VS. NoELS-Veh) (n=5-10). :



**Figure 6: Acute effect of HFD and social isolation for one week** on social recognition memory **in the adult animal.**

Animals at PND50 were divided into 4 groups: control diet and group housing (A-CD-Social), CD and isolation (A-CD-Isolation), High-fat diet and group housing (A-HFD-Social) and HFD and isolation (A-HFD-isolation). Immediately after the termination of the 7 days they were tested on social recognition memory test. ANOVA showed significant interaction between diet and housing [P<0.005]. Follow up analysis showed that only the CD-isolation was significantly different from the other groups while HFD+isolation showed intact recognition memory.

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