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HFD refeeding in mice after fasting impairs learning by activating caspase-1 in the brain

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Abstract

Background: Diets that include some aspect of fasting have dramatically increased in popularity. In addition, fasting reduces inflammasome activity in the brain while improving learning. Here, we examine the impact of refeeding a low-fat diet (LFD) or high-fat diet (HFD) after fasting.

Methods: Male wildtype (WT), caspase-1 knockout (KO) and/or IL-1 receptor 1 (IL-1R1) KO mice were fasted for 24 h or allowed ad libitum access to food (chow). Immediately after fasting, mice were allowed to refeed for 2 hrs in the presence of LFD, HFD or chow. Mouse learning was examined using novel object recognition (NOR) and novel location recognition (NLR). Caspase-1 activity was quantified in the brain using histochemistry (HC) and image analysis.

Results: Refeeding with a HFD but not a LFD or chow fully impaired both NOR and NLR. Likewise, HFD when compared to LFD refeeding increased caspase-1 activity in the whole amygdala and, particularly, in the posterior basolateral nuclei (BLp) by 2.5-fold and 4.6-fold,

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respectively. When caspase-1 KO or IL-1R1 KO mice were examined, learning impairment secondary to HFD refeeding did not occur. Equally, administration of n-acetylcysteine to fasted WT mice prevented HFD-dependent learning impairment and caspase-1 activation in the BLp. Finally, the free-fatty acid receptor 1 (FFAR1) antagonist, DC260126, mitigated learning impairment associated with HFD refeeding while blocking caspase-1 activation in the BLp.

Conclusions: Consumption of a HFD after fasting impairs learning by a mechanism that is dependent on caspase-1 and the IL-1R1 receptor. These consequences of a HFD refeeding on the BLp of the amygdala appear linked to oxidative stress and FFAR1.

Keywords

Fasting; Calorie restriction; Refeeding; Caspase-1; High-fat diet; Low-fat diet; Oxidative stress; Neuroimmunity; Learning; Memory; Amygdala

1.0 Introduction

As obesity has become a global scourge, a variety of dietary strategies have been touted as potential remedies [1]. Recently, intermittent fasting (IF) has gained traction due to its purported ability to reduce obesity and increase longevity [2,3]. Popular forms of IF include alternate-day fasting (ADF), time-restricted feeding (TRF), and, more recently, the 5:2 plan [4]. In general, these calorie restriction (CR)-like tactics appear to possess similar efficacy when used to combat and/or control weight gain, at least in mice [5]. Interestingly, certain obesity-associated brain-based comorbidities including anxiety [6,7], depression [8], and Alzheimer's disease [9] appear remedied or mitigated by IF. Furthermore, these salutary effects can extend intergenerationally as offspring of IF dams spend more time in the open area of an open field test (OFT), and offspring of IF fathers spend more time in the open arms of the elevated-plus maze [7,10].

An interesting consequence of fasting is that rodents tend to break a fast with high calorie gorging [11]. In humans, sex appears to govern the propensity to gorge, as men but not women seem to increase food intake following a fast [12]. This tendency to binge is likely reinforced by claims in media and popular culture that during the eating window of IF “you can eat what you want” [13]. Unsurprisingly, most work examining food consumption after IF has tended to focus on metabolic and cardiovascular endpoints like weight, body fat, blood glucose, resting heart rate and blood pressure [14]. Curiously, almost nothing is known about how refeeding after a fast impacts brain function.

As we have previously shown, acute fasting improves mouse memory while reducing caspase-1 activity in the brain [15]. Due to the well-recognized role of IL-1 β in learning and memory [16], activation of caspase-1 in the CNS appears critical to the impact of stress on cognition [17,18]. Necessary to the generation of secreted IL-1 β in the brain, is functional caspase-1 [19]. Produced as an inactive zymogen, procaspase-1 requires proximity-induced self-cleavage brought about by inflammasome assembly [20]. Non-infectious inducers of inflammasomes particularly relevant to the brain include: ionic flux, ER stress and mitochondrial reactive oxygen species (ROS) [20]. Known targets of caspase-1 are pro-IL-1 β , pro-IL-18 and gasdermin D [21].

Currently, the best described brain-associated morbidity linked to fasting is headache [22]. This form of secondary headache is believed to be connected to hypoglycemia [23]. In terms of food consumption, confusional states can rarely occur after eating as seen with postprandial (reactive) hypoglycemia [24] which most commonly afflicts those who have had surgery of the upper gastrointestinal track or Roux-en-Y gastric bypass surgery [25,26]. In mice, a high-fat diet (HFD) causes impaired novel object recognition (NOR) [27]. Therefore, we hypothesized that allowing fasted mice to refeed on a HFD would blunt the salutary effect of IF on memory/learning. In turn, given the importance of overnutrition to ROS generation [28], a HFD refeed should also increase the activity of brain-based caspase-1 since ROSs can activate the inflammasome [20].

2.0 Methods

2.1 Materials-

All reagents were purchased from Sigma-Aldrich (St Louis, MO) unless otherwise stated.

2.2 Animals-

Animal use was performed according to protocols approved by the Institutional Animal Care and Use Committee at the University of Illinois. Male C57BL/6J WT, as well as male caspase-1 KO and male IL1R1 KO mice on a C57BL/6J background, were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in-house. Mice were provided food and water *ad libitum* (*ad lib*) in standard shoebox cages (29.85cm × 18.4cm) unless otherwise stated. Animals were group housed (up to 8 per cage), then randomly assigned to treatment groups and moved to individual housing the day prior to the beginning of experimentation, unless otherwise noted. Housing temperature (72 °F) and humidity (45–55%) were controlled, and a 12/12h reversed dark-light cycle (2200–1000 h) was maintained. Animal behavior was video recorded using a Sony HDR-XR500V Night Shot capable video camera (Tokyo, Japan). Mice used were between 9 and 15 weeks of age. All behavior testing was completed under red lights in the dark cycle unless otherwise indicated. In total, 422 mice were used, mice were allocated to dietary treatments as follows: 128 (chow), 144 (LFD), and 150 (HFD).

2.3 Dietary treatment, food intake and body weight-

2 days prior to dietary treatment, mice were individually housed. Fasting for 24 hrs was accomplished by placing mice in a new cage without food (fasting arena) with *ad libitum* access to water. Refeeding entailed allowing fasted mice *ad libitum* access to either normal chow (Diet 7013, Envigo, Madison, WI; Supp. Fig. 1), LFD (D12450B, Research diets, New Brunswick, NJ; Supp. Fig.2) or HFD (D12492, Research Diets, New Brunswick, NJ; Supp. Fig. 3) for 2 hrs. For behavioral experiments, mice underwent the training phase for NOR or NLR prior to refeeding at 23.5 hrs of fasting. Food disappearance was measured as previously described [15]. In brief, food was placed in a dish inside the fasting arena and the reduction of weight of the dish + food before and after 2 hrs of refeeding was equated to food consumption. Mouse weight was recorded at the time points indicated using an Ohaus Adventurer Pro digital scale (Parsippany, NJ).

2.4 Injectables-

The caspase-1 inhibitor biotin-YVAD-CMK (YVAD) was purchased from AnaSpec (Fremont, CA), dissolved in DMSO and injected ICV at a dose of 1 ng/ μ L (1 μ L). The antioxidant and glutathione precursor N-acetyl cysteine (NAC) was dissolved in saline and injected IP at a dose of 50 mg/kg (200 μ L). The free fatty acid receptor 1 (FFAR 1) antagonist DC260126 was purchased from Tocris biosciences (Minneapolis, MN), dissolved in a 4% DMSO-saline and injected IP at a dose of 10 mg/kg (200 μ L). Control mice were injected with the appropriate dissolving agent at a volume matched to the bioactive. All injections were performed following the fasting period, and immediately before refeeding.

2.5 Novel Object Recognition-

NOR testing was utilized to test recognition memory and performed as previously described [15,29]. In brief, mice were placed in a learning/memory arena (26 cm $\times$ 48 cm $\times$ 21 cm) containing two identical objects placed at opposite ends of the arena. After this 30 minute learning session, mice were returned to their fasting arena and given access to the indicated food for 2 hrs. Object recognition was tested immediately after refeeding, 24 h after refeeding, and 48 h after refeeding by returning mice to a clean learning/memory arena where one of the objects (familiar object) was replaced by a new and different object (novel object). For 24 h and 48 h time points a new novel object was used at each time point. Object investigation was video recorded for 5 min and the video was evaluated using EthoVision XT 13 video tracking software (Noldus Information Technology, Leesburg, VA) by a blinded observer. Novel and familiar object investigation was quantified by dividing the time spent examining each object by the total object investigation time. Results are expressed as % novel object investigation.

2.6 Novel Location Recognition-

NLR testing was utilized to test spatial memory and performed as previously described [15,18]. In brief, mice were placed in a spatially-cued learning/memory arena (26 cm $\times$ 48 cm $\times$ 21 cm) containing two identical objects placed at the same end of the arena. After this 30 minute learning session, mice were returned to their fasting arena and given access to the indicated food for 2 hrs. Location recognition was tested immediately after refeeding by returning mice to a clean spatial-cued learning/memory arena where one of the objects (familiar position) was moved to the opposite end of the arena (novel location). Object investigation was video recorded for 5 min and the video was evaluated using EthoVision XT 13 video tracking software (Noldus Information Technology, Leesburg, VA) by a blinded observer. Novel and familiar location investigation was quantified by dividing the time spent examining each object by the total object investigation time. Results are expressed as % novel location investigation.

2.7 Locomotor activity-

Spontaneous locomotor activity was measured as we have previously described [29]. Mice were video recorded for 5 min in home cages modified for continuous video recording. A Sony Night Shot capable video camera (Minato-ku, Tokyo) was used for image capture, and

distance moved was quantified using Noldus Information Technology EthoVision XT 7 automated tracking software (Leesburg, VA).

2.8 Activated caspase-1 labeling-

Immediately following refeeding, mice were deeply anesthetized with ketamine/xylazine (80mg/kg/ 12mg/kg) then injected with 100 ng of biotin-YVAD-CMK ICV using a microinjection unit attached to a Kopf stereotaxic instrument (Tujunga, CA). Two hours following biotin-YVAD-CMK administration mice were euthanized using CO₂ and perfused with 30 mls of ice cold PBS followed by 30 mls of 4% paraformaldehyde. Brains were sectioned coronally at 1.5 and 3.5 mm from the interaural using a Mouse Brain Slicer (Zivic Instruments, Pittsburgh, PA). Slices were fixed in 4% paraformaldehyde for 24 hours, paraffin embedded and sectioned at 5 μ m. Fixed tissues were blocked with Peroxidized 1 (BioCare, Pacheco, CA), detected with SS horse radish peroxidase (BioCare), stained with liquid 3,3'-diaminobenzidine chromogen (BioCare) and counterstained with hematoxylin (BioCare) utilizing the BioCare IntelliPATH system (BioCare).

2.9 Image analysis-

Entire slides were imaged at 40 $\times$ with a NanoZoomer 2.0-HT (Hamamatsu, Bridgewater, NJ). For each amygdala analyzed, four regions of interest (ROIs) were delineated so as to cover the entire amygdala. Area of staining for each amygdala was determined using ImageJ (National Institute of Health) and an automated algorithm we developed and previously published [15]. In brief, area of staining was normalized to cell count and the results for each ROI were summed together. Statistical analysis was performed on the % area stained/100 cells value across all n. Due to variations in staining between replicate experiments that were performed on different days, results are expressed as % control. Representative images example the amygdala region quantified. For amygdala sub-nuclei % area stained, each sub-nuclei denoted underwent threshold imaging in ImageJ, as we have previously published [18]. Results are expressed as % area stained. Due to variations in staining between replicate experiments that were performed on different days, results are expressed as % control.

2.10 Statistics-

Data are expressed as mean $\pm$ SEM. Analysis was conducted using Sigma Plot 11.2 (Systat Software, Chicago, IL). To test for statistical differences, one-way and two-way ANOVAs were used. Pearson product moment correlation was used to determine correlation coefficients. Data were transformed where necessary to attain normality or equal variance. Post-hoc comparison used Tukey's test. Statistical significance was determined as $p < 0.05$.

3.0 Results

3.1 Mice refed a HFD eat more than twice the calories of chow- or LFD-refed mice.

Table 1 shows that mice refed chow, LFD, or HFD gain a similar amount of weight ($F=1.596$, $p=0.221$) and have comparable body weights ($F=0.14$, $p=0.87$) and locomotion ($F=3.095$, $p=0.066$) 2 hrs after refeeding. In contrast, mice refed chow or HFD consume 50% more food by weight than LFD-refed mice ($F=10.093$, $p<0.001$). Importantly, mice

refed a HFD consume 220% more calories than chow or LFD-refed mice ($F=24.473$, $p<0.001$).

3.2 Mice refed a HFD have impaired learning/memory.

Fig. 1A shows that mice refed a HFD lose the ability and/or desire to discern a novel object when compared to chow- and LFD-refed mice (% novel object investigation, chow vs. LFD vs. HFD, 64.99 ± 5.58 vs. 67.05 ± 1.04 vs. 51.99 ± 3.76 ; $F=5.546$, $p=0.012$). Figs. 1B & C show that this deficiency in learning/memory was present at 24 hrs (% novel object investigation, chow vs. LFD vs. HFD, 66.48 ± 1.64 vs. 67.81 ± 2.63 vs. 49.92 ± 3.50 ; $F=11.75$, $p<0.001$) but not 48 hrs post refeeding (% novel object investigation, chow vs. LFD vs. HFD, 49.95 ± 3.68 vs. 45.22 ± 3.34 vs. 46.69 ± 2.29 ; $F=0.58$, $p=0.569$). In Fig. 1A, NOR performance was negatively correlated to food disappearance during refeeding (Fig. 1D) ($r=-0.542$, $p=0.007$). Fig. 1E shows that mice refed a HFD lose the ability and/or desire to discern an object in a novel location when compared to chow- and LFD-refed mice (% novel location investigation, chow vs. LFD vs. HFD, 64.01 ± 3.83 vs. 58.68 ± 4.33 vs. 50.72 ± 2.44 ; $H=6.720$, $p=0.035$). This difference in NLR performance was negatively correlated to food disappearance (Fig. 1F) during refeeding ($r=0.464$, $p=0.022$). Importantly, total investigation did not differ between any groups in either the novel object task (Supp. Table 4; $F=0.487$, $p=0.621$) or novel location task (Supp. Table 4; $F=1.447$, $p=0.258$).

3.3 A HFD refeeding activates caspase-1 in the amygdala.

When compared to chow- or LFD-refed mice (Figs. 2A&B), HFD-refed mice had a 2-fold increase in caspase-1 activity in the whole amygdala (% control caspase-1 activity, chow vs. LFD vs. HFD, 100 ± 10.68 vs. 81.77 ± 18.02 vs. 186.61 ± 19.60 ; $F=10.595$, $p<0.001$). In turn, active caspase-1 was positively correlated with food disappearance (Fig. 2C) during refeeding ($r=0.594$, $p=0.000863$). When subnuclei of the amygdala were examined for refeeding-dependent caspase-1 activity, the BLP of HFD-refed mice was exclusively increased (Table 2).

3.4 Caspase-1 and IL1R1 KO mice are resistant to the memory/learning effects of a HFD refeed.

Fig. 3A shows that caspase-1 KO mice refed a HFD discern a novel object, while WT mice refed a HFD do not (% novel object investigation, WT chow vs. caspase-1 KO chow vs. WT LFD vs. caspase-1 KO LFD vs. WT HFD vs. caspase-1 KO HFD, 65.76 ± 2.76 vs. 62.58 ± 5.63 vs. 71.71 ± 2.58 vs. 70.39 ± 2.17 vs. 39.41 ± 4.36 vs. 63.01 ± 6.33 , significant genotype $\times$ diet interaction: $F=5.768$, $p=0.008$). Likewise, IL1R1 KO mice refed a HFD discern a novel object in contrast to WT mice refed a HFD (% novel object investigation, WT chow vs. IL1R1 KO chow vs. WT LFD vs. IL1R1 KO LFD vs. WT HFD vs. IL1R1 KO HFD, 56.54 ± 4.11 vs. 54.90 ± 4.54 vs. 67.63 ± 3.06 vs. 62.37 ± 2.68 vs. 46.23 ± 5.03 vs. 57.17 ± 2.84 , significant effect of diet: $F=6.45$, $p=0.003$).

3.5 NAC prevents learning/memory impairment and caspase-1 activation after a HFD refeed.

Fig. 4A demonstrates that mice administered NAC discern a novel object after a HFD refeed when compared to saline-administered mice refeed a HFD (% novel object investigation, saline chow vs. NAC chow vs. saline LFD vs. NAC LFD vs. saline HFD vs. NAC HFD, 64.95 ± 4.14 vs. 64.19 ± 2.59 vs. 62.60 ± 2.68 vs. 65.82 ± 1.82 vs. 45.79 ± 3.64 vs. 63.99 ± 3.54 , significant treatment $\times$ diet interaction: $F=5.034$, $p=0.011$). Impaired NLR performance after a HFD refeed was also corrected by NAC (Fig.4B) (% novel object investigation, saline chow vs. NAC chow vs. saline LFD vs. NAC LFD vs. saline HFD vs. NAC HFD, 64.68 ± 3.01 vs. 61.21 ± 4.76 vs. 66.36 ± 3.85 vs. 63.08 ± 4.98 vs. 45.45 ± 6.48 vs. 59.19 ± 2.83 , significant effect of diet treatment: $F=4.761$, $p=0.017$). Figs. 4C&D show that HFD-induced activation of caspase-1 in the whole amygdala is blocked by NAC (% control caspase-1 activity, saline chow vs. NAC chow vs. saline LFD vs. NAC LFD vs. saline HFD vs. NAC HFD, 100 ± 10.33 vs. 156.93 ± 40.83 vs. 138.19 ± 11.9 vs. 181.20 ± 30.16 vs. 356.96 ± 79.32 vs. 187.31 ± 69.47 , significant main effect of diet; $F=3.753$, $p=0.034$). Analysis of subnuclei demonstrate that NAC significantly reduced caspase-1 activity in the BLP of mice refeed a HFD (Table 3).

3.6 The FFAR1 antagonist DC260126 mitigates HFD refeed-induced learning/memory impairment.

When compared to DMSO administered mice, DC260126 treated mice (Figs. 5A&B) did not exhibit impaired NOR or NLR after a HFD refeed (% novel object investigation, DMSO chow vs. DC260126 chow vs. DMSO LFD vs. DC260126 LFD vs. DMSO HFD vs. DC260126 HFD, 61.46 ± 4.85 vs. 68.62 ± 3.66 vs. 72.35 ± 2.74 vs. 63.11 ± 2.22 vs. 51.51 ± 6.37 vs. 63.17 ± 3.46 , significant drug treatment $\times$ diet interaction; $F=3.967$, $p=0.027$), and novel object investigation, DMSO chow vs. DC260126 chow vs. DMSO LFD vs. DC260126 LFD vs. DMSO HFD vs. DC260126 HFD, 62.46 ± 3.09 vs. 58.83 ± 1.97 vs. 60.78 ± 3.17 vs. 60.03 ± 2.49 vs. 50.62 ± 4.11 vs. 64.54 ± 1.85 , significant drug treatment $\times$ diet interaction; $F=5.332$, $p=0.009$). Fig. 5C shows that FFAR1 antagonism did not significantly reduce caspase-1 activity in the whole amygdalae of HFD-refed mice (% control caspase-1 activity, DMSO chow vs. FFAR antagon chow vs. DMSO LFD vs. FFAR antagon LFD vs. DMSO HFD vs. FFAR antagon HFD, 100 ± 19.06 vs. 121.15 ± 15.37 vs. 123.96 ± 18.08 vs. 109.44 ± 18.31 vs. 224.07 ± 45.55 vs. 179.91 ± 49.69 , significant main effect of diet; $F=5.081$, $p=0.011$; Fig. 5C). In contrast, analysis of subnuclei demonstrate, that FFAR antagonism significantly reduced caspase-1 activity in both the BLP and ventral basolateral nuclei (BLV) of mice refeed a HFD (Table 4).

4.0 Discussion

Diets capable of inducing obesity are also associated with memory/learning impairment [30,31]. Even before clinical obesity is apparent, some types of overnutrition appear to adversely impact cognition [27]. This suggests that memory/learning and calorie intake are linked. Meta-inflammation is viewed as a potential connector of obesity and Alzheimer's disease [32–34], but this chronic overnutrition-associated low-grade inflammation is believed to impact cognition over many years [35–37]. Here we show that overnutrition

following IF in the form of a HFD-refeed can impair mouse memory/learning in as little as 2 hrs. The present model was used to model spontaneous ab-lib consumption of food following IF, which is germane to many current CR-based weight loss/control approaches [38].

In our previous work, we found that mice fasted for 24 hrs have improved cognition and decreased caspase-1 activity in the brain [15]. From a teleological standpoint, this hunger-associated enhancement in learning/memory is not surprising in that nutritional stress should be motivating and assist in foraging and/or food-finding behaviors. In contrast, postprandial fatigue [39] or, as it is known colloquially, “food coma” [40] appears to be a phenomenon with a less clear purpose or provenance. In humans, reduced mental performance is associated with the consumption of high-fat/low-carbohydrate meals [41–43]. Interestingly, IL-1 has been implicated in postprandial fatigue in humans in that administration of recombinant IL-1 receptor antagonist (IL-1RA) reduces the self-perception of sleepiness [39]. Although our data do not demonstrate a reduction in locomotor activity (aka physical fatigue), they do suitably support the concept of postprandial reduced mental performance. While refeeding of fasted mice increases serum IL-1 β and intraperitoneal macrophage production of IL-1 β [44], evidence that postprandial sleep occurs in animals is not clear. Work, however, in fruit flies indicates a metabolism/sleep connection, although it seems tied to salty or protein-rich foods rather than those high in fat [45].

As Table 1 shows, a HFD refeed did not cause a reduction in locomotor activity indicating that fatigue and/or somnolence was an unlikely source of HFD-dependent learning/memory impairment. Additionally, “fullness” is unlikely to be a significant factor, as chow-fed and HFD-fed mice appeared to eat a similar mass of food. Importantly, only a HFD refeed increased caspase-1 activity in the amygdala. As noted above, peripheral IL-1 β is boosted after mice are refeed after a 24 hr fast [44]. Fasting, however, reduces IL-1 α and β gene expression, and markedly increases upregulation of IL-1RA and the IL-1 decoy receptor, IL-1 receptor 2 (IL-1R2) [46,47]. Furthermore, fasted mice are relatively resistant to peripherally administered IL-1 β , as we have shown [46]. Therefore, while an increase in serum IL-1 β could be responsible for the impaired cognition observed, it seems unlikely in light of the sizeable increase in peripheral IL-1RA and IL-1R2 that is present post-fast [46]. More likely is a brain-based increase in IL-1 that is focally located at sites of increased caspase-1 activity. This may explain why increases in brain IL-1 β are nearly impossible to discern outside of conditions associated with significant neuroinflammation [48].

Reduced cognition and activation of caspase-1 in the amygdala correlated with food disappearance during refeeding. These findings support the concept that overnutrition is detrimental to brain health [49]. Increasingly, caloric load and/or macronutrient composition appear to regulate the inflammasome or its components [47]. As example, rats fed a high-fructose diet demonstrate up-regulation of the NLRP3 inflammasome while developing obesity, high serum triglycerides and elevated blood non-esterified fatty acids (NEFAs) [50]. As Fig.3 demonstrates, caspase-1 KO and IL-1R1 KO mice were resistant to HFD-dependent learning/memory impairment when compared to LFD. In IL-1R1 KO mice, chow fed mice were not statistically different than HFD-fed mice. These results appear to underscore recent work illustrating the disadvantage of using a undefined diet as a control

when a matched defined diet control is available [51]. Since functional caspase-1 is necessary for the generation of secreted IL-1 β [52–54] and the IL-1R1 is required for IL-1 signaling [55–59], our data support the concept that localized caspase-1-dependent maturation of IL-1 β may be responsible for the postprandial cognitive impairment observed. These new data and our previous work [15,46,47,60,61] also point to the importance of caspase-1 activation in the brain as causative to impaired learning/memory.

Unlike caspase-1 activation in the brain secondary to handling stress [18], refeeding-dependent activation of caspase-1 appears limited in its area of distribution (Tables 2–4). On a macro scale, the whole amygdala showed a significant increase in caspase-1 activation after a HFD refeed (Fig.2), with the basolateral amygdala groups (BLA) showing discernible caspase-1 activity when compared to cortical-like groups and the centromedial groups (data not shown). However, when specific nuclei of the BLA were analyzed, only the BLp demonstrated a diet-dependent change in caspase-1 activity (Table 2). Interestingly, the BLp connects to the ventral hippocampal CA1 (vCA1) participating in positive emotions [62] that can potentiate spatial memory [63]. Since a HFD refeed impairs NLR (a test of spatial memory [29]), it appears that activation of caspase-1 in the BLp may negatively impact circuits that link emotion to memory. This may also begin to explain why some individuals categorized in a binge-eating status display reduced mental acuity [64]. In turn, animals display anxiety-like behavior and cognitive impairment after binge eating [65].

Non-infectious activators of the inflammasome particularly relevant to the brain include: ionic flux, lysosomal rupture, ER stress and mitochondrial ROS [20]. Whether macronutrients directly activate inflammasomes or act through the above well-known danger signals is not clear [47]. Consumption of HFDs and high calorie loads from fatty acids induces oxidative stress [27,66,67] and peroxidation products [68]. In turn, oxidative stress can precipitate cognitive dysfunction [69]. Fig 4 and Table 3 show that administration of NAC prevents HFD-dependent learning/memory impairment and activation of caspase-1 in the BLp. This suggests that ROS may be causative. Previous work has demonstrated that oxidative stress activates brain inflammasomes, and that antioxidants reduce the subsequent activation of caspase-1 [70]. However, oxidative stress as a generic activator of caspase-1 in the brain is not universal. As example, hypoxia triggers caspase-1 activation even though administration of NAC blocks hypoxia-dependent depression of the brain glutathione to glutathione disulfide (GSH/GSSG) ratio [61].

We have previously shown that mice administered IP palmitic acid exhibit impaired NOR 24 hrs after injection [71]. Fig.5 shows that DC260126 mitigates HFD-dependent learning/memory impairment suggesting that FFAR1 is important to the postprandial cognitive dysfunction observed. Interestingly, DC260126 did not reduce whole amygdala caspase-1 activity due to a HFD refeed (Fig.5C), but it did decrease it in the BLp and BLV (Table 4). Relative to other subnuclei of the amygdala, the BLp contains reduced numbers of somatostatin-positive GABA-reactive neurons [72]. In addition, the BLp is important to reward conditioning [73] and stimuli that exert a significant influence on behavior including scent, water, sucrose and peanut oil [74]. Compared to the BLV, the BLp has stronger connections to the vCA1 region of the hippocampus [63]. Currently, it is unknown if the BLV is functionally similar to the BLp, but it does contain FFAR1 mRNA in neurons [75].

In general, FFAR1 is widely expressed in the brain, and its absence increases brain TNF- α mRNA and insulin resistance [76]. Therefore, FFAR1-dependent activation of caspase-1 may be part of a neuroprotective mechanism as is caspase-1 activation during excitotoxin damage to the retina [77].

Taken together, our results suggest that excessive calorie consumption after a fast induces memory impairments through a pathway reliant on oxidative stress. Although highly speculative, a potential pathway by which a HFD could activate caspase-1 in the brain is as follows: increased dietary fat is a well-known inducer of oxidative stress [66,68], and overnutrition impairs molecular redox-buffering systems which increase mitochondrial ROS production [78]. Oxidative stress, as with other non-lethal cellular injuries, triggers docosahexaenoic acid (DHA) release from astrocytes [79,80]. DHA is a potent activator of FFAR1 in the brain [81], and, as a Gq/11 linked receptor, FFAR1 activates phospholipase C (PLC) prompting increases in cytoplasmic calcium [82,83]. This bi-cellular mechanism is necessary because neurons lack the enzymes required to synthesize DHA on their own [84]. In turn, intracellular calcium elevations in neurons opens big potassium (BK) channels [85] leading to neuronal depolarization [86] and the activation of inflammasomes [87]. This proposed pathway is akin to how extracellular adenosine depolarizes neurons and activates caspase-1 during hypoxia [88]. However, there are important limitations to this work including: 1) Restricting most experimentation to a single time point of refeeding; 2) ad libitum refeeding as opposed to calorie-limited refeeding; 3) A reliance on NOR and NLR as the only tests of memory/learning; and 4) indirect assessment of IL-1 β functionality through caspase-1 KO and IL-1R1 KO mice.

Finally, this work highlights how just 2 hrs of overnutrition following a fast can impair cognition. It has intriguing implications for those interested in or already embarked on an IF regimen, since triggering the symptoms of food coma seem adversely associated with compliance and the feeling of well-being ascribed to IF by advocates [89].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

HC	Histochemistry
5-HIAA	5-hydroxyindoleacetic acid
BLA	Basolateral amygdala
BK	Big potassium
CR	Calorie restriction

DHA	Docosahexaenoic acid
FFAR 1	Free fatty acid receptor 1
HFD	High-fat diet
IF	Intermittent fasting
LFD	Low-fat diet
IL-1R1	IL-1 receptor 1
IL-1R2	IL-1 receptor 2
IL-1RA	IL-1 receptor antagonist
IP	intraperitoneal
KO	Knockout
NAC	N-acetyl cysteine
NOR	Novel object recognition
NLR	Novel location recognition
PLC	Phospholipase C
BLp	Posterior basolateral amygdala nuclei
BLV	Ventral basolateral amygdala nuclei
WT	Wild type

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Highlights

- After a 24 hr fast, a 2 hr high-fat diet (HFD) refeed impairs cognition in mice
- Refeeding a HFD after a fast increases caspase-1 activation in the amygdala of mice
- After a fast, HFD-dependent caspase-1 activation is localized to the posterior basolateral nuclei of the amygdala
- NAC and FFAR1 antagonism prevent HFD-dependent memory impairment and caspase-1 activation post fast in mice

Figure 1. Mice refed a HFD have impaired learning/memory.

(A) Mice were fasted for 24 hr then refed chow (Chow), low-fat diet (LFD), or high-fat diet (HFD) for 2 hr. Novel object recognition (NOR) was performed immediately post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; $n=6-9$ /group. (B) NOR performed 24 h post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; $n=6-9$ /group. (C) NOR performed 48 h post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; $n=6-9$ /group. (D) NOR was correlated with kcal consumption during refeeding. A significant correlation was observed ($p=0.007$); $n=23$ total. (E) Mice were refed as in (A). Novel location recognition (NLR) was performed immediately post refeeding. Results are expressed as mean $\pm$ SEM % novel location investigation; $n=8$ /group. (F) NLR was correlated with kcal consumption during refeeding. A significant correlation was observed ($p=0.022$); $n=24$ total. Values in (A, B, C, and E) without a common superscript are significantly different ($p<0.05$).

Figure 2. A HFD refeeding activates caspase-1 in the amygdala.

(A) Mice were fasted for 24 hr then re-fed chow (Chow), low-fat diet (LFD), or high-fat diet (HFD) for 2 hr. Immediately post-refeeding, caspase-1 activity was measured in the amygdala. Results are expressed as mean $\pm$ SEM % control; $n=11-13$ /group. Values without a common superscript are significantly different ($p<0.05$). (B) Representative images (40 $\times$) from (A). (C) Caspase-1 activity was positively correlated with kcal consumption during refeeding. A significant correlation was observed ($p=0.00454$); $n=36$ total.

Figure 3. Caspase-1 and IL1R1 KO mice are resistant to the memory/learning effects of a HFD refeed.

(A) Wild-type (WT) and caspase-1 KO (Casp-1 KO) mice were fasted for 24 hr then refed chow (Chow), low-fat diet (LFD), or high-fat diet (HFD) for 2 hr. Novel object recognition (NOR) was performed immediately post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; n=5–6/group. (B) WT and IL-1R1 KO (IL1R1 KO) mice were refed as in (A). NOR was performed immediately post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; n=8–12/group. Values in (A and B) without a common superscript are significant different (p<0.05).

Figure 4. NAC prevents learning/memory impairment and caspase-1 activation after a HFD refeed.

Mice were fasted for 24 hr then administered n-acetylcysteine (NAC) at 50 mg/kg or saline vehicle control (Sal). Mice were refed chow (Chow), low-fat diet (LFD), or high-fat diet (HFD) for 2 hr. (A) Novel object recognition (NOR) or (B) novel location recognition (NLR) were performed immediately post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; $n=6-9$ /group or mean $\pm$ SEM % novel location investigation; $n=5-6$ /group, respectively. (C) Mice were fasted, administered NAC and refed as in (A/B). Immediately post-refeeding, caspase-1 activity was measured in the amygdala. Results are expressed as mean $\pm$ SEM % control; $n=4-8$ /group. (D) Representative images (40 $\times$) from (C). For (A-C) values without a common superscript are significantly different ($p<0.05$).

Figure 5. The FFAR1 antagonist DC260126 mitigates HFD refeed-induced learning/memory impairment.

Mice were fasted for 24 hr then administered DC260126 at 10 mg/kg or 4% DMSO vehicle control (DMSO). Mice were refeed chow (Chow), low-fat diet (LFD), or high-fat diet (HFD) for 2 hr. (A) Novel object recognition (NOR) or (B) novel location recognition (NLR) were performed immediately post refeeding. Results are expressed as mean $\pm$ SEM % novel object investigation; $n=7-8$ /group and mean $\pm$ SEM % novel location investigation; $n=8$ /group, respectively. (C) Mice were fasted, administered DC260126 and refeed as in (A/B). Immediately post-refeeding, caspase-1 activity was measured in the amygdala. Results are expressed as mean $\pm$ SEM % control; $n=6-8$ /group. (D) Representative images (40×) from (C). For (A-C) values without a common superscript are significantly different ($p<0.05$).

Table 1.

Body weight, body weight change, food disappearance, locomotor activity of mice 2 hours after being refed Chow, LFD, or HFD

	Chow	LFD	HFD
Body weight, g	22.86 ± 0.59	21.33 ± 0.51	22.06 ± 0.70
Body weight change, % gain	5.44 ± 1.16	6.08 ± 0.81	6.17 ± 1.14
Food disappearance, g	1.41 ± 0.18 ^a	0.93 ± 0.12 ^b	1.86 ± 0.17 ^a
Food disappearance, kcal	4.38 ± 0.56 ^a	3.58 ± 0.45 ^a	9.77 ± 0.87 ^b
Locomotor activity, cm	2226.26 ± 177.23	2701.38 ± 183.86	2254.32 ± 92.48

Results are expressed as mean ± SEM n=7–14.

Values with a different superscript are different ($P < 0.05$).

Table 2.

Caspase-1 activity (% area) in amygdala subnuclei.

	Amygdala Subnuclei						
	LaDL	LaVL	LaVM	BLp	BLa	BMP	BLV
Chow	0.217 ± 0.06	0.24 ± 0.05	0.21 ± 0.12	1.00 ± 0.53 ^{a,b}	0.64 ± 0.40	0.82 ± 0.37	0.47 ± 0.19
LFD	1.10 ± 0.65	0.54 ± 0.27	0.67 ± 0.25	0.55 ± 0.12 ^a	0.61 ± 0.24	1.17 ± 0.40	0.43 ± 0.16
HFD	0.37 ± 0.274	0.89 ± 0.30	0.94 ± 0.37	2.53 ± 0.59 ^b	1.49 ± 0.50	1.87 ± 0.47	1.24 ± 0.33

Results are expressed as mean % area stained ± SEM. N=7–11/group.

Results with a different superscript are significantly different (p<0.05).

LaDL, dorsolateral lateral amygdala nuclei; LaVL, ventrolateral lateral amygdala nuclei; LaVM, ventromedial lateral amygdala nuclei; BLp, posterior basolateral amygdala nuclei; BLa, anterior basolateral amygdala nuclei; BMP, posterior basomedial amygdala nuclei; BLV, ventral basolateral amygdala nuclei.

Table 3.

Caspase-1 activity (% area) in amygdala subnuclei.

		Amygdala Subnuclei						
		LaDL	LaVL	LaVM	BLp	BLa	BMP	BLV
Chow	Sal	0.37 ± 0.31	0.37 ± 0.31	0.14 ± 0.08	0.51 ± 0.26 ^a	0.85 ± 0.37	1.17 ± 0.62	1.16 ± 0.55
	NAC	0.37 ± 0.15	0.52 ± 0.24	0.62 ± 0.32	0.52 ± 0.26 ^a	0.52 ± 0.22	0.44 ± 0.16	0.59 ± 0.32
LFD	Sal	0.31 ± 0.14	0.28 ± 0.09	0.77 ± 0.62	0.33 ± 0.09 ^a	0.73 ± 0.54	1.22 ± 0.45	0.56 ± 0.27
	NAC	0.60 ± 0.22	0.50 ± 0.19	1.03 ± 0.40	0.97 ± 0.38 ^a	0.92 ± 0.45	0.77 ± 0.22	0.91 ± 0.23
HFD	Sal	1.87 ± 0.66	2.80 ± 1.34	2.13 ± 0.96	2.50 ± 0.62 ^b	1.98 ± 0.66	1.49 ± 0.56	2.53 ± 0.94
	NAC	0.70 ± 0.53	0.52 ± 0.22	0.65 ± 0.40	0.73 ± 0.39 ^a	0.73 ± 0.36	0.54 ± 0.25	1.02 ± 0.28

Results are expressed as mean % area stained ± SEM. N=4–7/group.

Results with a different superscript are significantly different (p<0.05).

LaDL, dorsolateral lateral amygdala nuclei; LaVL, ventrolateral lateral amygdala nuclei; LaVM, ventromedial lateral amygdala nuclei; BLp, posterior basolateral amygdala nuclei; BLa, anterior basolateral amygdala nuclei; BMP, posterior basomedial amygdala nuclei; BLV, ventral basolateral amygdala nuclei.

Table 4.

Caspase-1 activity (% area) in amygdala subnuclei.

		Amygdala Subnuclei						
		LaDL	LaVL	LaVM	BLp	BLa	BMP	BLV
Chow	DMSO	1.65 ± 0.46	2.18 ± 0.99	1.76 ± 0.56	2.33 ± 0.81 ^{a,b}	2.47 ± 0.71	3.01 ± 1.12	3.03 ± 1.45 ^{a,b}
	FFAR Antag	1.63 ± 0.84	1.12 ± 0.60	1.84 ± 1.01	1.36 ± 0.41 ^{a,b}	2.03 ± 0.98	2.54 ± 1.08	2.37 ± 0.92 ^{a,b}
LFD	DMSO	1.06 ± 0.39	2.58 ± 1.13	1.79 ± 0.82	1.56 ± 0.47 ^a	1.66 ± 0.93	2.77 ± 0.98	2.53 ± 0.88 ^a
	FFAR Antag	1.06 ± 0.38	1.42 ± 0.46	1.41 ± 0.43	1.44 ± 0.35 ^a	1.48 ± 0.28	2.26 ± 0.45	1.60 ± 0.47 ^a
HFD	DMSO	2.15 ± 0.43	4.04 ± 0.72	2.99 ± 0.63	4.00 ± 0.75 ^b	4.09 ± 0.66	5.49 ± 1.40	7.13 ± 2.31 ^b
	FFAR Antag	3.14 ± 1.70	1.81 ± 0.70	1.36 ± 0.34	2.23 ± 0.71 ^a	1.62 ± 0.44	3.41 ± 0.84	3.55 ± 0.99 ^a

Results are expressed as mean % area stained ± SEM. N=5–8/group.

Results with a different superscript are significantly different (p<0.05).

LaDL, dorsolateral lateral amygdala nuclei; LaVL, ventrolateral lateral amygdala nuclei; LaVM, ventromedial lateral amygdala nuclei; BLp, posterior basolateral amygdala nuclei; BLa, anterior basolateral amygdala nuclei; BMP, posterior basomedial amygdala nuclei; BLV, ventral basolateral amygdala nuclei.