Immunity & Ageing

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Obesity-associated memory impairment and neuroinflammation precede widespread peripheral perturbations in aged rats

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Abstract

Background Obesity and metabolic syndrome are major public health concerns linked to cognitive decline with aging. Prior work from our lab has demonstrated that short-term high fat diet (HFD) rapidly impairs memory function via a neuroinflammatory mechanism. However, the degree to which these rapid inflammatory changes are unique to the brain is unknown. Moreover, deviations in gut microbiome composition have been associated with obesity and cognitive impairment, but how diet and aging interact to impact the gut microbiome, or how rapidly these changes occur, is less clear. Thus, our study investigated the impact of HFD after two distinct consumption durations: 3 months (to model diet-induced obesity) or 3 days (to detect the rapid changes occurring with HFD) on memory function, anxiety-like behavior, central and peripheral inflammation, and gut microbiome profile in young and aged rats.

Results Our data indicated that both short-term and long-term HFD consumption impaired memory function and increased anxiety-like behavior in aged, but not young adult, rats. These behavioral changes were accompanied by pro- and anti-inflammatory cytokine dysregulation in the hippocampus and amygdala of aged HFD-fed rats at both time points. However, changes to fasting glucose, insulin, and inflammation in peripheral tissues such as the distal colon and visceral adipose tissue were increased in young and aged rats only after long-term, but not short-term, HFD consumption. Furthermore, while subtle HFD-induced changes to the gut microbiome did occur rapidly, robust age-specific effects were only present following long-term HFD consumption.

Conclusions Overall, these data suggest that HFD-evoked neuroinflammation, memory impairment, and anxiety-like behavior in aging develop quicker than, and separately from the peripheral hallmarks of diet-induced obesity.

Keywords Cytokines, High fat diet, Gut microbiome

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Background

Obesity and metabolic syndrome increase the risk for cognitive decline, Alzheimer's disease, and other dementias [1, 2]. Consumption of a Western diet, characterized by high saturated fats and refined carbohydrates, is a major driver of obesity and metabolic syndrome and is also strongly linked to memory impairment across many species, ranging from early vertebrates like zebrafish [3, 4], to mammals, including mice [5, 6], rats [7–10], and humans [11]. Despite the high degree of conservation across species, the mechanism(s) linking obesity and metabolic syndrome to cognitive decline are still unclear [12, 13]. For example, preclinical rodent studies demonstrate that consumption of a Western diet is linked to cognitive dysfunction, yet this effect was independent of long-term metabolic perturbations [2, 14, 15]. In humans, consumption of a Western diet is associated with reductions in hippocampal volume regardless of metabolic syndrome, insulin resistance, or hypertension status [16–19]. These findings highlight a critical need to understand the unknown mechanisms linking obesity and/or Western diet intake to cognitive decline.

Western diets may directly lead to declines in cognitive function through low-grade inflammation [1]. This is supported by emerging data in aging populations, who experience chronic inflammation and immunological priming in both the periphery and the brain [20–22], demonstrating they may be especially vulnerable to cognitive deficits from pro-inflammatory Western diets [23–25]. Our previous work provided initial evidence that consumption of a high fat diet (HFD) leads to a rapid (within 3 days) memory impairment in aged, but not young adult, rats without changes to fasting glucose or insulin [9, 10], suggesting that diet-induced memory impairment precedes changes to systemic insulin sensitivity. HFD does, however, lead to a rapid neuroinflammatory response in the aged hippocampus and amygdala and blocking this neuroinflammatory response prevents memory impairments, thus establishing neuroinflammation as a causal factor linking HFD to rapid cognitive decline [9]. The degree to which these rapid inflammatory changes are unique to the brain is unknown. Another likely contributor to the impact of diet and/or metabolic syndrome on the aging brain and behavior is the gut microbiome. Prior studies have reported that aging [26–28] and obesity [29–31] independently induce significant dysregulation of gut microbiota, although less is known about how these two variables interact to impact the gut microbiome, or how quickly these changes may occur.

Our study investigates the impact of HFD after two distinct consumption durations: 3 months (to model diet-induced obesity) or 3 days (to detect the rapid changes occurring with HFD) on memory function, anxiety-like behavior, central and peripheral inflammation, and gut microbiome in young and aged rats. We hypothesize that while long-term HFD will lead to a widespread inflammatory response, including in the brain, and significant changes to the gut microbiome, these peripheral changes will not be necessary for the HFD-induced neuroinflammatory response and subsequent behavior alterations.

Materials and methods

Subjects

Young adult (3–5 months) and aged (22–24 months) male F344xBN F1 rats obtained from the National Institute on Aging rodent colony managed by Charles River were used. F344xBN F1 rats are particularly useful for the study of aging and aging-associated conditions as aged rats of this strain remain relatively healthy and show good cognitive function at baseline. Female rats of this strain were not consistently available from this or any other vendor at the time these studies were completed. Thus, any data from the current study in males should not be generalized to females and, moving forward, additional experiments should rigorously address similar questions in females, which we have already begun to do [32, 33]. Young adult male rats weighed approximately 350 g and aged male rats weighed approximately 550 g upon arrival at our facility. Age- and condition-matched rats were housed 2 to a cage (52 L × 30 W × 21H, cm). The animal colony was maintained at 22 ± 1 ∘C on a 12-h light/dark cycle (lights on at 07:00 h). All subjects were allowed ad libitum access to food and water and were given at least 1 week to acclimate to colony conditions before experimentation began. For the long-term HFD study, subjects were used for both behavioral tests and tissue collection. In the short-term HFD study, separate cohorts were used for open field, contextual fear conditioning, and tissue collection to ensure all data corresponded to the 3-day timepoint. All experiments were conducted in accordance with protocols approved by the Ohio State University Animal Care and Use Committees. Every effort was made to minimize the number of animals used and their suffering.

Diets

Subjects were randomly assigned to either continue consuming their regular grain-based chow (Inotiv TD.8640, energy density of 3.0 kcal/g; 29% calories from protein, 54% from carbohydrates, and 17% from fat), or a high-fat diet (HFD), which is an adjusted calorie HFD (Inotiv TD.06414, energy density of 5.1 kcal/g; 18.3% calories from protein, 21.4% from carbohydrates, and 60.3% from fat), for 3 months (long-term) or 3 days (short-term) (Table 1) (Fig. 1). As is the nature of adjusted calorie diets, significantly increasing fat content requires

Table 1 Macronutrient composition of diets

Diet	Chow	High Fat Diet
Energy density	3.0 kcal/g	5.1 kcal/g
Calories from:		
Protein	29%	18.3%
Carbohydrates	54%	21.4%
Fat	17%	60.3%
% of fat from:		
Saturated	19%	36%
Monounsaturated	25%	41%
Polyunsaturated	56%	23%
Added sugar:		
Sucrose	0 g/kg	90 g/kg
Maltodextrin	0 g/kg	160 g/kg

a compensatory reduction in protein and/or carbohydrate content. This is not possible to the same extent with grain-based diets. Thus, the two diets could not be matched on all other macronutrients. Therefore, we cannot rule out a role for changes in these other macronutrients based on the present experimental design. Rats in the long-term experiment were fed their assigned diet for 12 weeks before behavioral testing began and remained on that diet for the duration of the experiment. Rats in this experiment were weighed (in g) at the same time of day (9:00 – 10:00 am) once per week. Subjects in the short-term experiment were age-matched to those in the long-term experiment at the time of behavior/tissue collection and fed chow or HFD for three days before resuming regular chow for the remainder of the experiment. In this experiment, rats were switched back to chow to ensure any effects observed were a consequence of HFD-induced impairment in memory consolidation. These rats were weighed (in g) at the same time of day (9:00 – 10:00 am) each day of the diet.

Open field test

Open field test (OFT) was used to assess anxiety-like behaviors, including center avoidance and freezing. Rearing, a form of exploratory behavior that is reliant on the hippocampus [34], was also evaluated. Rats were placed one at a time into one corner of an opaque 24″ W × 24″ L × 15″ H arena for 10 min under standard overhead lighting (approximately 300 lx). The floor of the arena was lightly covered in corncob bedding. An overhead camera was used to record each session. The ANY-maze video tracking system was used to measure the percentage of time spent in the center (middle third of chamber) of the arena. Freezing and rearing behaviors were manually scored by observers blind to the experimental conditions using the recorded task videos. Freezing is a rat's dominant fear response, characterized by complete suppression of behavior, including immobility and shallow breathing, and autonomic changes such as increased heart rate and piloerection [35]. In these experiments, freezing was defined as the absence of all visible movement, except for respiration. Rearing was counted each time a rat stood only on its hind legs, raising its forelimbs off the ground. Chambers were cleaned with water and 70% ethanol and allowed to dry before each animal was tested.

Contextual fear conditioning

Contextual fear conditioning was used to assess hippocampus- and amygdala-dependent long-term memory function. This test was chosen because we have validated its use in assessing memory impairment in both young adult and aging F344xBN F1 rats following a variety of insults. Rats were conditioned using the Coulbourn Instruments Habitest Modular System. Each conditioning chamber (12″ W × 10″ D × 12″ H) had two solid metal walls and two walls made of Plexiglass, with an audio speaker and a house light mounted on the ceiling. A foot shock could be delivered through a removable grid-shock floor. The rods were wired to a shock generator for eight unique shock outputs. Each conditioning chamber was placed inside an isolation cubicle (23″ W × 20″ D × 24″ H). Chambers were cleaned with water, 70% ethanol, and allowed to dry before each animal was conditioned or tested.

Following either 3mo or 3d on their diet assignment (Fig. 1), rats were taken two at a time from their home cage and each was placed in a separate conditioning chamber. Rats were allowed to explore the chamber for 2 min before the onset of a 15 s tone (76 dB), which was followed immediately by a 2 s foot-shock (1.5 mA). To assess obvious signs of lethargy or sickness, locomotion was evaluated during conditioning, prior to the tone-shock pairing. Immediately after the termination of the shock, rats were removed from the chamber and returned to their home cage. Four days later, rats were tested for fear of the conditioning context, a hippocampus-dependent task [36], and then for fear of the tone, an amygdala-dependent task [37, 38]. For the contextual memory test, rats were placed in the exact context in which they were conditioned for 6 min and were observed and scored for freezing behavior. For the auditory cued-fear test, rats were placed in a unique context consisting of a differently shaped and sized chamber with wire walls and cob bedding, and dim lighting. Rats were scored for freezing behavior for 3 min, then the tone was activated and freezing behavior was scored for an additional 3 min. Rats were scored manually using a

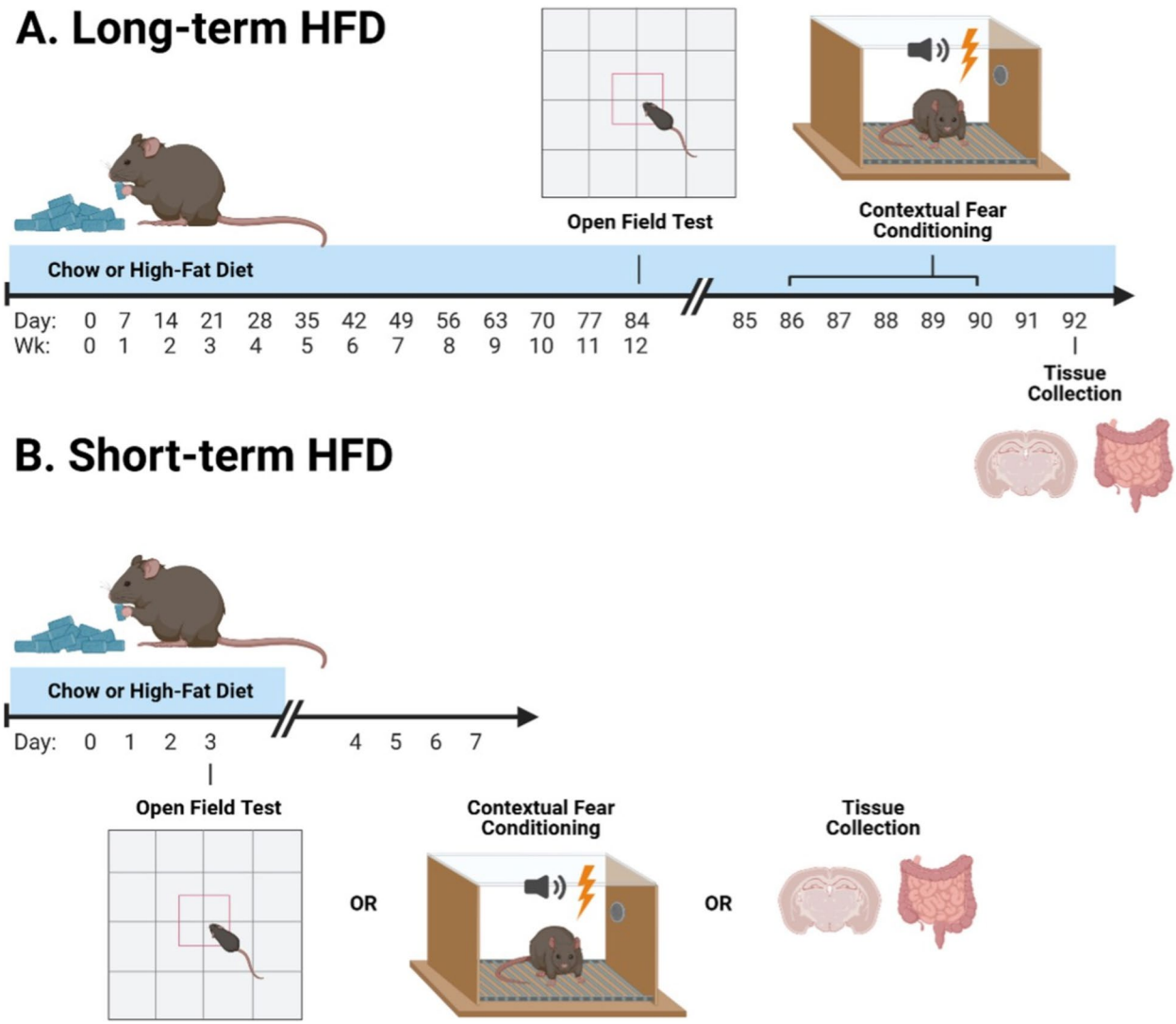

Fig. 1 Experimental timeline. **A** For the long-term HFD experiment, young adult and aged rats were fed HFD for 12 weeks. At the end of the 12th week, behavioral testing began: OFT was conducted on day 84 and two days later CFC began. On day 86, rats underwent the conditioning phase of CFC, and four days later their contextual and cued memory was tested. Two days after behavioral testing, tissue was collected. **B** For the short-term HFD experiment, young adult and aged rats were fed HFD for 3 days. On the 3rd day, rats were switched back to chow, and subjected to either OFT, CFC, or sacrifice for tissue collection. Different cohorts were used for each outcome to ensure the observed effects were due to just 3 days of HFD. For CFC, which is a multi-day test, rats were conditioned on day 3 and four days later their contextual and cued memory was assessed

time sampling procedure as described previously [39]. Subjects were evaluated as either freezing or not freezing in real-time, at the instant the sample was taken by three observers blind to treatment conditions. Scoring began 10 s after rats were placed in the chamber and continued every 10 s for the entire 6 min observation period. Scores were averaged, and inter-rater reliability exceeded 95% for all experiments. Data are presented as the percentage of time scored as freezing during the 6 min period.

Glucose & insulin measurements

Morning blood glucose after 2 h of fasting was measured from whole blood samples using a commercial (Bayer Contour) glucometer. Fasting plasma insulin was measured using an enzyme-linked immunosorbent assay (ELISA) kit for rat insulin from Abnova (Taiwan) with a detection range of 0 µLU/mL – 140 µLU/mL and a sensitivity of < 5 µLU/mL.

Tissue collection

For biochemical assays at the 3mo timepoint, rats were euthanized following the full battery of behavioral testing. For the 3d timepoint, tissue was collected 2 h after the tone-shock pairing phase of the contextual fear conditioning paradigm (as described above), a time that coincides with memory consolidation.

For all experiments, rats were given a lethal dose of sodium pentobarbital (65 mg/kg, i.p.) and transcardially perfused with ice-cold saline (0.9%) for 3 min to remove peripheral immune leukocytes from the CNS vasculature. Complete necropsies of each subject were conducted for all experiments to insure there were no tumors, cysts, or tissue abnormalities. Following perfusion, brains were rapidly extracted, placed on ice, and hippocampus and amygdala were dissected. At the same time, peripheral tissues, including the distal colon, ileum, visceral adipose tissue, and distal colon contents were collected. All samples were flash frozen in liquid nitrogen and stored at −80 °C until processing.

Tissue processing for protein analysis

In preparation for protein assays, hippocampus, amygdala, colon, ileum, or adipose tissue was manually sonicated in 0.3 mL buffer for 20 s using an ultrasonic cell disrupter (ThermoFisher Scientific). Sonication buffer contained 50 mM Tris base and an enzyme inhibitor cocktail that included 100 mM amino-n-caproic acid, 1 mM EDTA, 5 mM benzamidine HCl, 0.2 mM phenylmethyl sulfonyl fluoride, and a cOmplete™ Mini protease inhibitor cocktail tablet (Millipore Sigma). Following sonication, samples were centrifuged at 10,000 rpm at 4 °C for 10 min and supernatants were transferred into a clean tube. Bradford protein assays were performed on all samples (prior to the first freeze) to determine total protein concentrations. Samples were divided into 60 µL aliquots and frozen at −80 °C until assays were performed.

Multiplex enzyme-linked immunosorbent assays

Protein levels of interleukin (IL)−1β, IL-6, IFNγ, IL-4, IL-10, and tumor necrosis factor (TNF) were determined using a commercially available rat-specific multiplex ELISA (Meso Scale Discovery, MD, USA). The assays were performed according to the manufacturer instructions. Values of all cytokines were determined and normalized to total protein ($n = 6–8$/group) and expressed as pg/ug of total protein. Detection limits for each analyte is as follows: IL-1β: < 5 pg/mL, IL-6: 21 pg/mL, TNF: < 5 pg/mL, IFNγ < 5 pg/mL, IL-4: < 5 pg/mL, and IL-10: 16.4 pg/mL.

Microbiome analysis

Fecal samples were collected from rats fed a long-term or short-term HFD and stored at −80 °C. DNA extraction was performed using a QIAamp Fast DNA Stool Mini Kit following manufacturer's instructions, with slight modifications as previously described [40]. Briefly, stool was incubated for 45 min at 37 °C in lysozyme buffer (22 mg/ml lysozyme, 20 mM TrisHCl, 2 mM EDTA, 1.2% Triton-x, pH 8.0), then bead-beat for 150 s with 0.1 mm zirconia beads. Samples were incubated at 95 °C for 5 min with InhibitEX Buffer, then incubated at 70 °C for 10 min with Proteinase K and Buffer AL. Following this step, the QIAamp Fast DNA Stool Mini Kit isolation protocol was followed, beginning with the ethanol step. DNA was quantified with the Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) using the dsDNA Broad Range Assay Kit. After extraction and DNA quality assurance by gel electrophoresis, library construction was completed using a Fluidigm Access Array system in the Functional Genomics Unit of the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign. After library construction, 250 bp of the V4 region of the 16SrRNA gene were amplified and sequenced at the WM Keck Center for Biotechnology at the University of Illinois using an Illumina MiSeq2000. The V4 region of the 16S rRNA gene was amplified using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-CCG YCAATTYMTTTRAGTTT-3'). PCR reactions were conducted in triplicate and resulting amplicons were pooled.

Illumina libraries were generated from the pooled amplicons and paired-end (2 × 250 nt) sequencing was performed with an Illumina MiSeq System. After sequencing and barcode trimming, raw sequence data (FASTQs) underwent quality control using DADA2, trimming low-quality bases with a cutoff Phred score > 30. DADA2 was used for denoising, merging paired-end reads, and inferring ASVs (amplicon sequence variants). ASVs were mapped to SILVA rRNA database (138-SSU) with QIIME2. Alpha diversity metrics, including Faith's PD and Shannon, were calculated to assess within-sample diversity. Taxa abundance data were transformed using the center log ratio (CLR) method to mitigate compositional biases and improve interpretability. Negative binomial regression models were employed to explore interactions between age and diet. Statistical Analysis and graphs were completed with MicrobiomeAnalyst [41].

Statistical analysis

For all experiments, $n = 6–8$ rats/group were utilized throughout the study, based on statistical power established by our previous work. Statistical analyses were

performed using Prism v.10 software. Outliers, as determined by Grubb's test, were removed prior to statistical tests. For any given analysis, a maximum of two samples were deemed outliers and were excluded from the data set. Data met the requirements for parametric analysis, based on Shapiro–Wilk tests for normality and Q-Q plots. Two-way ANOVAs were run for the experiments that had a 2×2 factorial design. In the case of significant interactions, Tukey's multiple comparisons *posthoc* tests were run. The threshold for significance was set as $\alpha = 0.05$.

Results

Behavioral effects

Long-term and short-term HFD induce anxiety-like behavior in aged male rats

In the OFT, there were main effects of age ($F_{(1,24)} = 20.34$, $p = 0.0001$), diet ($F_{(1,24)} = 16.52$, $p = 0.0004$), and an age x diet interaction ($F_{(1,24)} = 23.15$, $p < 0.0001$; Fig. 2A, D) for time spent in the center. A pairwise analysis showed that aged chow-fed rats spent more time in the center of the OFT compared to young adult chow-fed controls ($p < 0.0001$) (Fig. 2A). However, long-term HFD caused a significant reduction in time spent in the center in aged rats compared to chow-fed aged controls ($p < 0.0001$) (Fig. 2A). A 2-way ANOVA of freezing behavior revealed main effects of age ($F_{(1,25)} = 11.95$, $p = 0.0020$) and diet ($F_{(1,25)} = 8.711$, $p = 0.0068$), and an interaction effect ($F_{(1,25)} = 6.489$, $p = 0.0174$; Fig. 2B). Pairwise comparisons showed that aged HFD-fed rats spent significantly more time freezing compared to HFD-fed young adult and chow-fed aged controls ($p = 0.0028$ and $p = 0.0024$, respectively (Fig. 2B). For rearing, a main effect of diet ($F_{(1,25)} = 9.870$, $p = 0.0043$) and an age x diet interaction effect ($F_{(1,25)} = 7.188$, $p = 0.0128$; Fig. 2C) were observed. Aged, HFD-fed rats had significantly fewer rearing episodes compared to HFD-fed young adult and chow-fed aged controls, evidenced by pairwise analysis ($p = 0.0016$ and $p = 0.0284$, respectively). In all three of these measures, young adult rats, regardless of diet condition, did not differ from each other ($p > 0.05$).

These anxiety-like behavioral changes emerge rapidly after HFD onset, as similar effects were observed following short-term HFD. At this timepoint, 2-way ANOVA assessment showed main effects of age ($F_{(1,19)} = 29.10$, $p < 0.0001$) and diet ($F_{(1,19)} = 22.21$, $p = 0.0001$), as well as an interaction effect ($F_{(1,19)} = 22.70$, $p < 0.0001$) (Fig. 2E, H). Consistent with the long-term cohort, aged chow-fed rats spent significantly more time in the central part of the OFT chamber compared to chow-fed young controls ($p < 0.0001$). This aging-related anxiolytic effect was prevented by HFD, as aged HFD-fed rats spent significantly less time in the center than their chow-fed controls ($p < 0.0001$) (Fig. 2E). For freezing behavior, a main effect of age ($F_{(1,19)} = 19.67$, $p = 0.0003$), diet ($F_{(1,19)} = 26.29$, $p < 0.0001$), and an age x diet interaction effect ($F_{(1,19)} = 13.03$, $p = 0.0019$) were observed (Fig. 2F). Pairwise analyses revealed that aged HFD-fed rats froze significantly more than their young adult and chow-fed counterparts ($p < 0.0001$ and $p = 0.0001$, respectively). In the rearing measure, a 2-way ANOVA showed main effects of age ($F_{(1,19)} = 4.582$, $p = 0.0455$) and diet ($F_{(1,19)} = 7.989$, $p = 0.0108$), and an age x diet interaction effect ($F_{(1,19)} = 5.102$, $p = 0.0358$; Fig. 2G). As with long-term HFD, short-term HFD resulted in aged rats having significantly fewer rearing episodes compared to their young adult and chow-fed counterparts ($p = 0.0078$ and $p = 0.0314$, respectively). Again, in all three of these measures, young adult rats, regardless of diet condition, did not differ from each other ($p > 0.05$).

Long-term and short-term HFD induce memory impairments in aged male rats

Following long-term HFD consumption, aged rats experienced significant contextual and cued-fear memory deficits. For contextual memory, a 2-way ANOVA revealed main effects of age ($F_{(1,25)} = 5.961$, $p = 0.0220$) and diet ($F_{(1,25)} = 24.82$, $p < 0.0001$), as well as an age x diet interaction ($F_{(1,25)} = 10.46$, $p = 0.0034$), to decrease time spent freezing, indicating impaired memory (Fig. 3A). Pairwise comparisons revealed that HFD-fed aged rats performed significantly worse on the contextual memory task than HFD-fed young adult and chow-fed aged controls ($p < 0.0001$ and $p = 0.0041$, respectively; Fig. 3A). Similar effects were observed in the cued-fear memory task. A 2-way ANOVA showed that 3 months of HFD induced a significant effect of age ($F_{(1,24)} = 17.26$, $p = 0.0004$) and diet ($F_{(1,24)} = 16.16$, $p = 0.0005$) to reduce time spent freezing, as well as an interaction effect ($F_{(1,24)} = 5.029$, $p = 0.0344$; Fig. 3B). Aged rats fed HFD exhibited significant cued-fear memory impairment compared to HFD-fed young adult and chow-fed aged controls ($p = 0.0014$ and $p = 0.0011$, respectively; Fig. 3B).

To assess how rapidly these memory impairments occur following initiation of a HFD, we assessed contextual and cued-fear memory function following short-term (just 3 days) consumption of HFD. In the contextual memory task, short-term HFD resulted in main effects of age ($F_{(1,26)} = 35.33$, $p < 0.0001$) and diet ($F_{(1,26)} = 76.21$, $p < 0.0001$), as well as an age x diet interaction ($F_{(1,26)} = 5.731$, $p = 0.0242$), to impair performance on the test (Fig. 3C). Pairwise analysis showed that aged rats fed short-term HFD were significantly impaired to young adult and chow-fed controls ($p < 0.0001$ and $p < 0.0001$, respectively; Fig. 3C). On the cued-fear task, short-term HFD induced a main effect

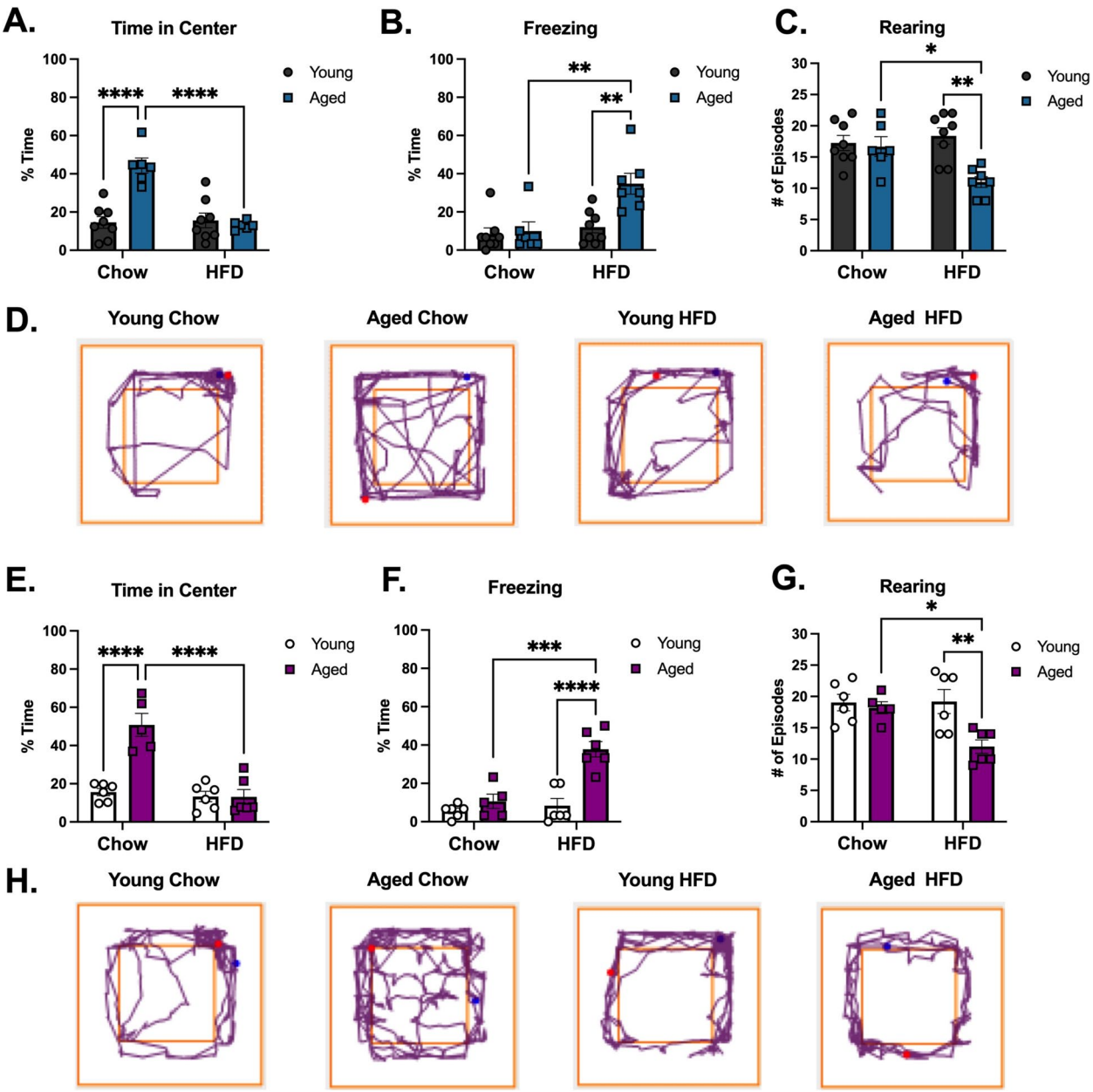

Fig. 2 Measures of anxiety-like behavior via the open field test (OFT) in young and aged rats following long- and short-term HFD consumption**.** Percentage of time spent in center during OFT following (**A**) long and (**E**) short-term HFD consumption. Percentage of time spent freezing during OFT following (**B**) short and (**F**) long-term HFD consumption. The number of rearing episodes during the OFT following (**C**) long and (**G**) short-term HFD consumption. Representative plots of routes traced during OFT following (**D**) long and (**H**) short-term HFD consumption. *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$; ****$p < 0.0001$

of age ($F_{(1,26)} = 4.284$, $p = 0.0485$) and an age x diet interaction ($F_{(1,26)} = 5.139$, $p = 0.0319$) to reduce time spent freezing (Fig. 3D). Aligning with our previous findings, aged rats fed short-term HFD were significantly impaired compared to young adult and chow-fed controls ($p = 0.0284$ and $p = 0.0303$, respectively; Fig. 3D). This finding that just 3 days of HFD induces long-term contextual and cued-fear memory deficits replicates the behavioral findings associated with long-term HFD consumption, indicating that HFD rapidly induces memory impairment which persists throughout diet consumption.

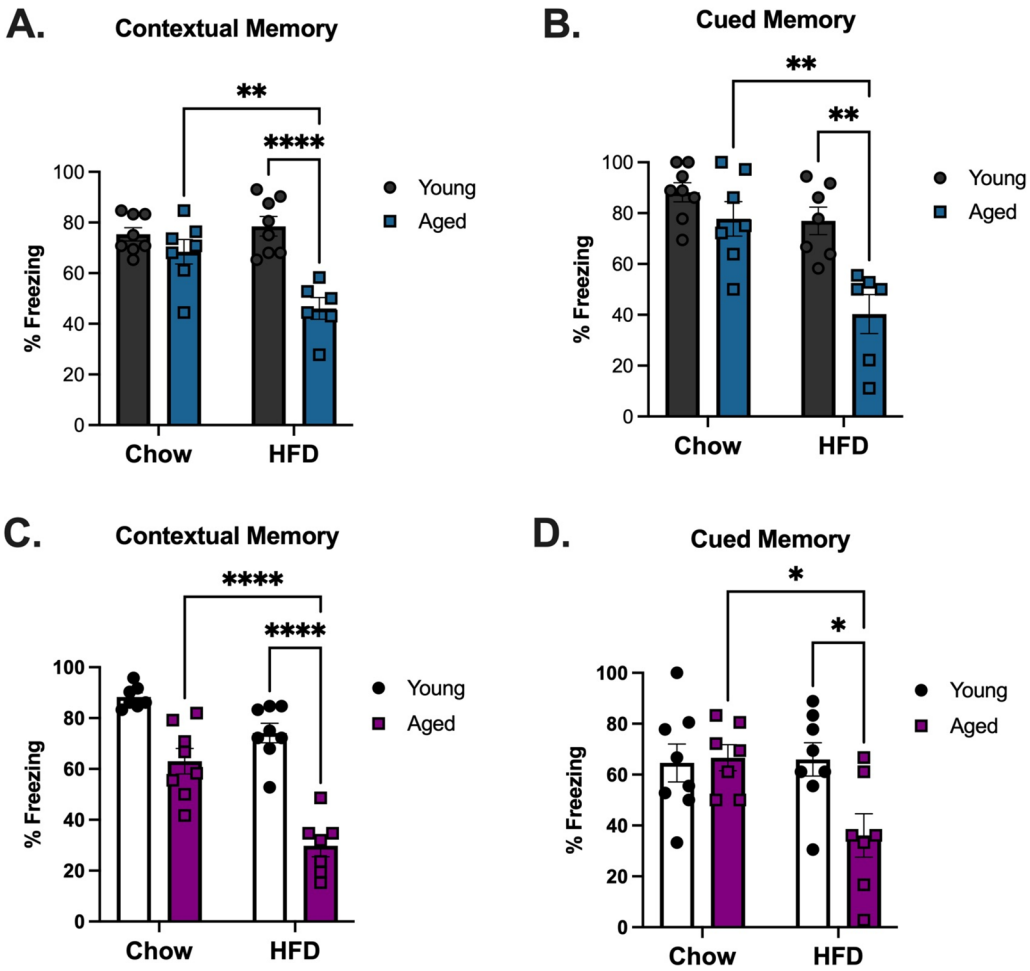

Fig. 3 Long-term memory function assessed via contextual and cued-fear conditioning following long-term and short-term HFD consumption. Freezing behavior during the testing phase for hippocampal-dependent contextual memory following (**A**) long-term and (**C**) short-term HFD consumption. Freezing behavior during the testing phase for amygdala-dependent cued-fear memory following (**B**) long-term and (**D**) short-term HFD consumption. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$

Neuroinflammation

Long-term HFD alters inflammatory cytokines in the hippocampus and amygdala of young and aged rats

Following 3 months of either chow or HFD, we measured changes in protein levels of pro and anti-inflammatory cytokines in the hippocampus and amygdala. In the hippocampus, there was a significant age x diet interaction in all cytokines except IL-6 (IL-1β: $F_{(1,25)} = 4.441$, $p = 0.0453$; TNF: $F_{(1,26)} = 4.544$, $p = 0.0425$; IL4: $F_{(1,25)} = 6.132$, $p = 0.0204$; IL10: $F_{(1,24)} = 7.211$, $p = 0.0129$; IFNγ $F_{(1,26)} = 4.443$, $p = 0.0448$; Fig. 4A-F). Post hoc analyses of these cytokines revealed a significant decrease in cytokine levels in HFD-fed aged rats compared to HFD-fed young adult rats (IL-1β, IL4, and IL10: $p < 0.01$; TNF, IFNγ $p < 0.05$). Results of IL-6 showed a main effect of age ($F_{(1,26)} = 10.26$, $p = 0.0036$; Fig. 4B), such that aged rats had decreased IL-6, relative to young, regardless of diet condition.

In the amygdala, there was also a significant age x diet interaction for all cytokines except IL-6 and IL-10 (IL-1β: $F_{(1,24)} = 4.270$, $p = 0.0497$; TNF: $F_{(1,21)} = 11.65$, $p = 0.0029$; IL-4: $F_{(1,24)} = 5.169$, $p = 0.0322$; IFNγ: $F_{(1,21)} = 12.37$, $p = 0.0018$; Fig. 5). Pairwise comparisons indicated that aged HFD-fed rats had higher levels of IL-1β ($p < 0.05$; Fig. 5A), TNF ($p < 0.005$; Fig. 5C) and IL-4 ($p < 0.01$; Fig. 5D) compared to young HFD-fed rats. Moreover, aged HFD-fed rats had elevated IFNγ levels relative to all other groups ($p < 0.005$ – 0.05; Fig. 5F). There was no significant differences across groups for IL-6 and IL-10 in the amygdala.

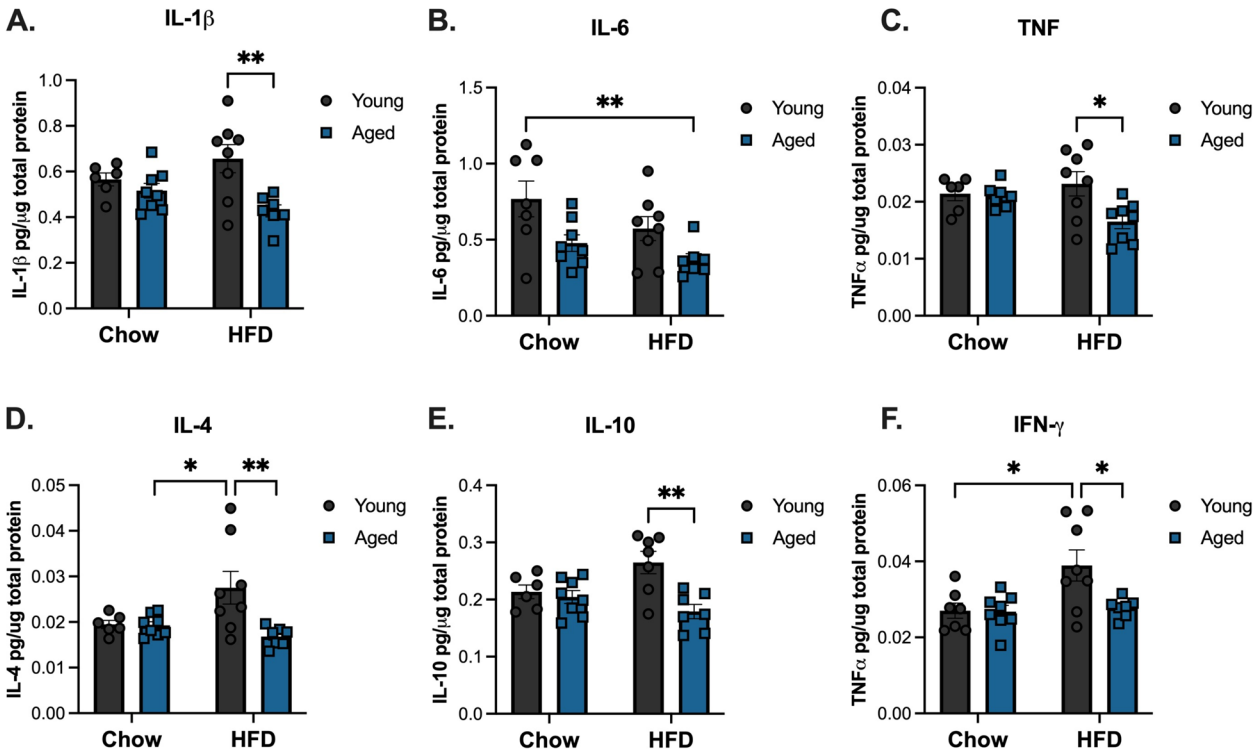

Fig. 4 Inflammatory protein concentration in the hippocampus following long-term HFD consumption in young and aged rats. Levels of (**A**) IL-1β, (**B**) IL-6, (**C**) TNF, (**D**) IL-4, (**E**) IL-10, and (**F**) IFNγ in the hippocampus. *$p < 0.05$, **$p < 0.01$

Short-term HFD alters inflammatory cytokines in the hippocampus and amygdala of aged rats

The concentration of the same proteins were measured in the hippocampus and amygdala of young and aged rats following short-term consumption of either chow or HFD. In the hippocampus, there was a significant age x diet interaction for all cytokines except TNF and IFNγ (IL-1β: $F_{(1,28)} = 7.957$, $p = 0.0087$; IL-6: $F_{(1,27)} = 10.68$, $p = 0.0029$; IL-4: $F_{(1,28)} = 4.656$, $p = 0.0397$; IL-10: $F_{(1,28)} = 4.424$, $p = 0.0446$; Fig. 6). Posthoc analyses of each of these cytokines demonstrated a significant increase in cytokines in aged rats fed a HFD, relative to all other groups ($p < 0.005 - 0.05$). While there was not a significant interaction, there was a main effect of age for TNF levels ($F_{(1,28)} = 11.97$, $p = 0.0017$; Fig. 6C), as aged rats displayed higher levels than young. There was also a main effect of diet on IFNγ concentration in the hippocampus ($F_{(1,27)} = 12.51$, $p = 0.0015$; Fig. 6F), with HFD-fed rats showing increased levels relative to chow-fed controls.

Additionally, we observed dysregulated cytokines in the amygdala following short-term HFD. There was a significant age x diet interaction for IL-1β ($F_{(1,28)} = 6.003$, $p = 0.0208$; Fig. 7A), IL-6 ($F_{(1,19)} = 11.04$, $p = 0.0036$; Fig. 7B), and IFNγ ($F_{(1,25)} = 9.787$, $p = 0.0044$; Fig. 7F). Tukey's posthoc tests revealed young HFD-rats had less IL-1β than aged chow- and HFD-fed rats. Furthermore, young HFD-fed rats had increased IL-6 ($p < 0.01$) and IFNγ ($p < 0.05$) levels compared to young chow-fed rats, with IFNγ concentration also being increased in young HFD-fed rats relative to aged HFD-fed rats ($p < 0.05$). For TNF in the amygdala, there was a main effect of age ($F_{(1,28)} = 4.326$, $p = 0.0468$; Fig. 7C), as aged rats had increased TNF levels relative to young. Lastly, we observed a main effect of diet on amygdala IL-10 concentration ($F_{(1,28)} = 5.008$, $p = 0.0334$; Fig. 7E), with IL-10 levels being increased in HFD-fed rats.

Metabolic effects

Long-term, but not short-term, HFD consumption induces hyperglycemia and visceral adipose tissue inflammation in young and aged rats

Because obesity and metabolic syndrome are associated with significant weight gain, hyperglycemia, and increased inflammation in adipose tissue, we evaluated these metrics at two distinct time points during diet consumption. For the long-term HFD cohort, there was a main effect of diet ($F_{(1,25)} = 65.42$, $p < 0.0001$; Fig. 8A) on the percent of body weight gained during the study, such that HFD-fed rats gained more weight than chow-fed rats ($p < 0.0001$). There was also a main effect of age

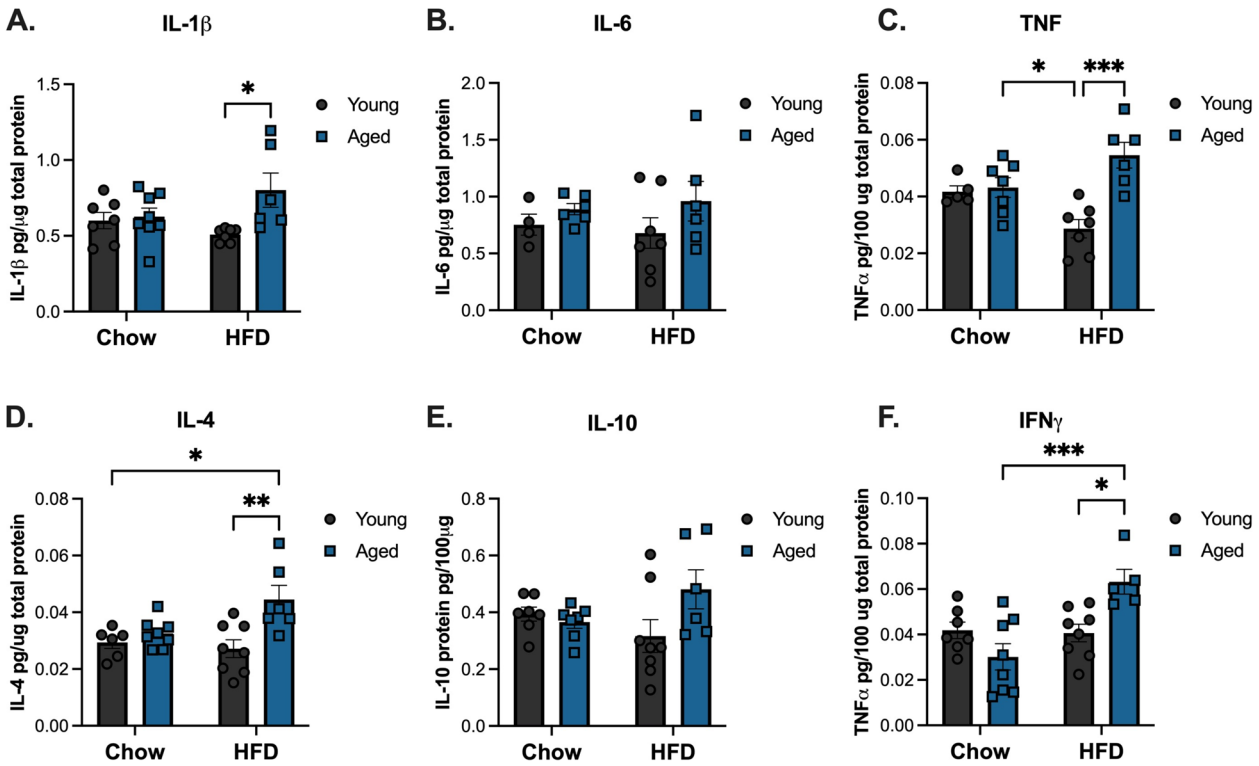

Fig. 5 Inflammatory protein concentration in the amygdala following long-term HFD consumption in young and aged rats. Levels of (**A**) IL-1β, (**B**) IL-6, (**C**) TNF, (**D**) IL-4, (**E**) IL-10, and (**F**) IFNγ in the amygdala. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

($F_{(1,25)} = 27.67$, $p < 0.0001$; Fig. 8A) on the percent of body weight gain, where aged rats gained less during the course of the 3mo study, relative to young rats ($p < 0.0001$). For short-term HFD consumption, there was an age x diet interaction ($F_{(1,19)} = 4.936$, $p = 0.0386$; Fig. 8E) for the percent of body weight gained. Post hoc analysis revealed that aged, HFD-fed rats gained more weight than young, HFD-fed rats and young and aged chow-fed controls during the 3 days on the diet ($p < 0.05$). There were no differences in percent weight gain between young and aged chow-fed rats ($p > 0.05$).

Following long-term HFD consumption, there was a main effect of diet on serum levels of fasting glucose ($F_{(1,27)} = 15.680$, $p = 0.0005$; Fig. 8B) and insulin ($F_{(1,27)} = 8.436$, $p = 0.0073$; Fig. 8C) in both young and aged rats. Importantly, there were no differences in fasting glucose or insulin levels in young or aged rats following short-term HFD consumption (Fig. 8F&G). Similarly, only long-term HFD consumption resulted in increased inflammation in visceral adipose tissue, as indicated by increased IL-1β in both young and aged rats that consumed HFD, relative to chow-fed controls ($F_{(1,22)} = 54.300$, $p < 0.0001$; Fig. 8D). There were no differences in visceral adipose tissue IL-1β in young or aged rats following short-term HFD consumption (Fig. 8H).

Gut inflammation

Long-term, but not short-term, HFD induces a proinflammatory phenotype in the aged colon

Given the intricate gut-brain connection and prior work demonstrating HFD and obesity are each associated with gut inflammation [42–44], we measured various pro and anti-inflammatory cytokines in the colon and ileum tissues following both long-term and short-term HFD consumption in young and aged rats. At the 3-month timepoint (long-term) in the colon, a two-way ANOVA indicated a main effect of diet ($F_{(1,24)} = 11.27$, $p = 0.0026$; Fig. 9A) on IL-1β such that HFD significantly increased levels compared to chow-fed rats, regardless of age. There was also a significant age x diet interaction for IL-6 ($F_{(1,24)} = 5.490$, $p = 0.0277$; Fig. 9B), IL-4 ($F_{(1,25)} = 6.339$, $p = 0.0186$; Fig. 9D), and IL-10 ($F_{(1,24)} = 5.053$, $p = 0.0340$; Fig. 9E). For all three cytokines, post hoc analysis revealed that young HFD-fed rats had increased levels of each cytokine, relative to all other groups ($p < 0.05$). There were no differences in colon TNF levels across any condition ($p > 0.05$). In the ileum, there was a main effect of age for IL-1β ($F_{(1,24)} = 5.053$, $p = 0.0161$; Fig. 10A). Specifically, aged rats had lower levels of IL-1β than young rats ($p < 0.05$). There were no impacts of HFD on the quantified cytokines in the ileum at the 3-month timepoint

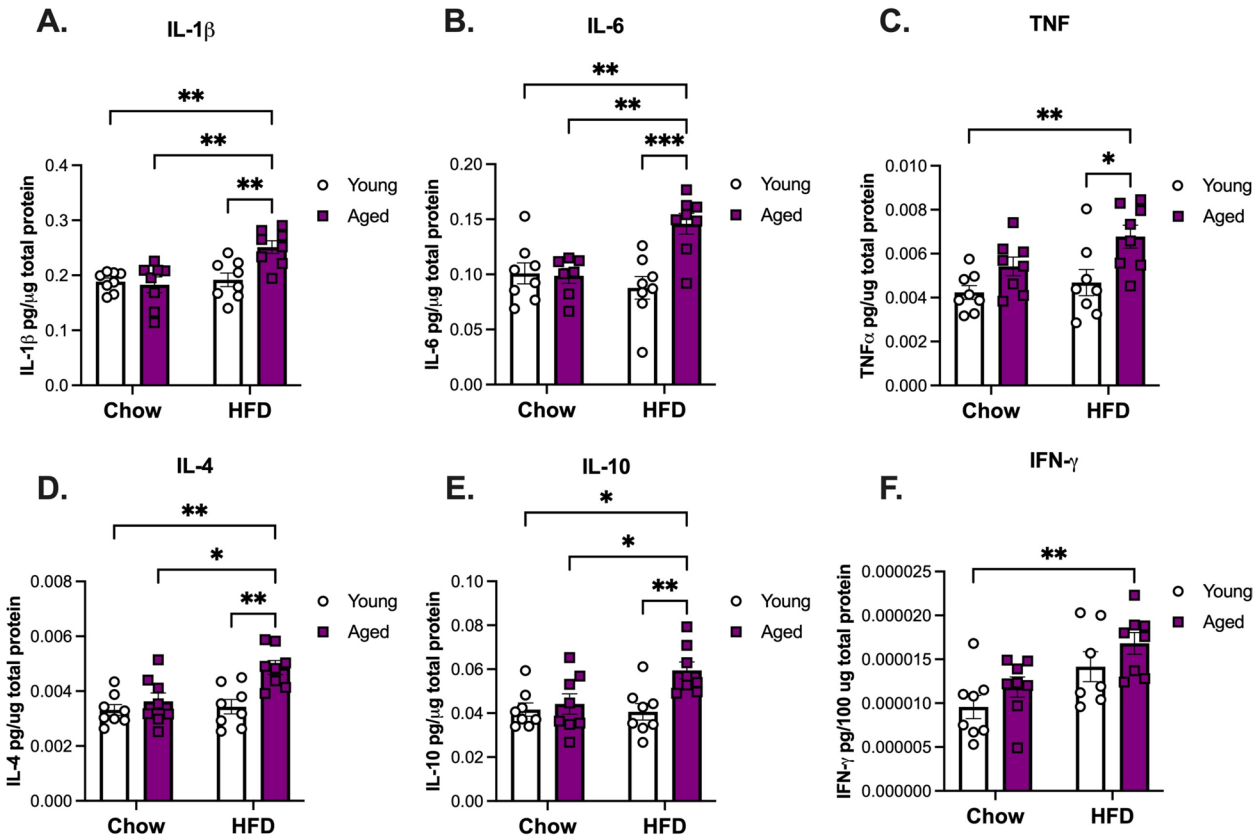

Fig. 6 Inflammatory protein concentration in the hippocampus following short-term HFD consumption in young and aged rats. Levels of (**A**) IL-1β, (**B**) IL-6, (**C**) TNF, (**D**) IL-4, (**E**) IL-10, and (**F**) IFNγ in the hippocampus. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

(Fig. 10). Importantly, there was no impact of short-term HFD on either colon or ileum cytokines that were measured ($p > 0.05$ for all effects; Figs. 11 and 12, respectively).

Microbiome

Aged rat microbiome is more sensitive to a long-term HFD

We next investigated how long-term HFD impacted the gut microbiota of both young and aged rats. Long-term HFD reduced alpha diversity across both ages, yet older rats exhibited a more pronounced decrease in microbial richness as compared to their young counterparts (Faith's PD—Fig. 13A and Shannon index—Fig. S1A $p < 0.05$). HFD also induced robust compositional shifts in the gut microbiome (Fig. S1B). Some of these HFD-induced compositional shifts were independent of age, including HFD-induced expansion of *Lactococcus sp,* and *Blautia* sp that occurred in both age groups. Moreover *Turicibacter sp.* and *Clostridia*_UCG-014 were downregulated by HFD independent of age (Fig. 13B). However, aged mice also exhibited unique microbiome responses to HFD (Fig. S1C). Notably, long-term HFD led to more pronounced expansion of *Ruminococcus torques, Enterococcus* sp, *Clostridium innocuum,* and *Eubacterium siraeum* in aged rats vs. young rats (Fig. 13C, Age x HFD Two-factor negative binomial (NEGBIN) $p < 0.05$).

Short-term HFD rapidly alters gut microbiome irrespective of age

As compared to long-term HFD, short-term (3 days) HFD did not change microbial richness as measured by Faith's PD. Aged rats exhibited lower alpha-diversity compared to their younger counterparts, but only in the chow group (Fig. 14A). Short-term HFD elicited changes to microbial taxa (Fig. S2C), yet most of these effects occurred independent of age. For example, HFD increased Lactococcus and *Colidexbacter*, and reduced *Lachnospiraceae* UCG-006 and *Prevotellaceae* NK3B31 (Fig. 14B and Fig. S2B).

Spearman correlational analysis in the aged group revealed associations between microbial taxa and neuroinflammation indices after only 3 days on HFD. Most prominent was *Lachnospiraceae* UCG-006, which was downregulated by age and HFD, and negatively associated with amygdala and hippocampus TNF levels (Fig. 14D). In addition, *Prevotellaceae* NK3B31, downregulated by HFD, negatively correlated with several

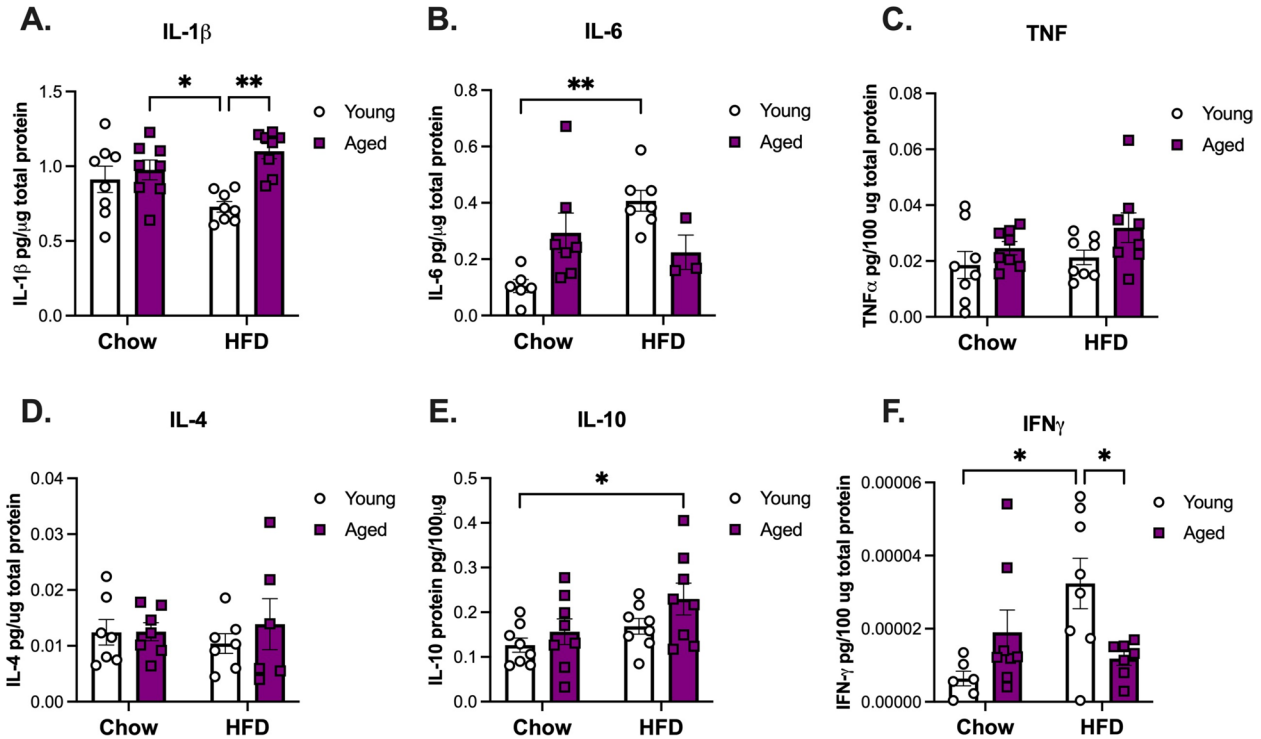

Fig. 7 Inflammatory protein concentration in the amygdala following short-term HFD consumption in young and aged rats. Relative expression of (**A**) IL-1β, (**B**) IL-6 (**C**) TNF (**D**) IL-4 (**E**) IL-10, (**F**) IFNγ levels in the amygdala. ****$p < 0.0001$

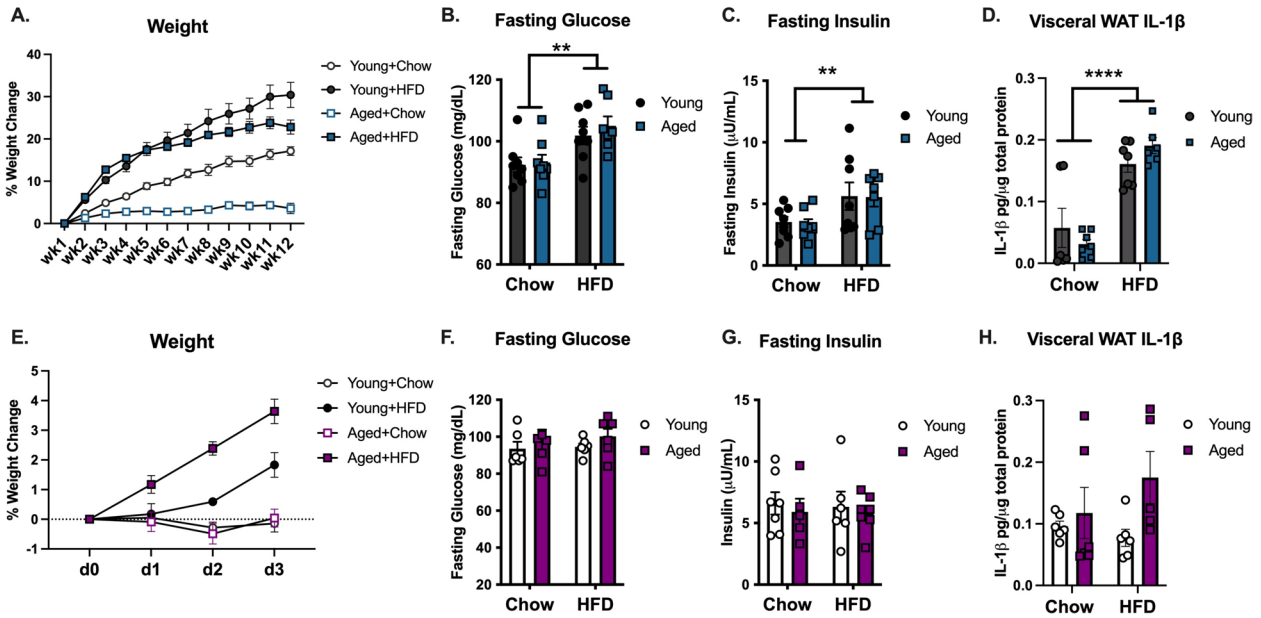

Fig. 8 Measurement of body weight, circulating metabolic markers, and visceral adipose tissue inflammation in young and aged rats following long- and short-term HFD. **A** Percent of body weight gained by young and aged rats following 3mo of HFD consumption. Serum levels of (**B**) fasting glucose and (**C**) fasting insulin and **D**) protein levels of IL-1beta in visceral adipose tissue in young and aged rats following 3mo of HFD consumption. **E** Percent of body weight gained by young and aged rats following 3d of HFD consumption. Serum levels of (**F**) fasting glucose and (**G**) fasting insulin and (**H**) protein levels of IL-1beta in visceral adipose tissue in young and aged rats following 3d of HFD consumption. **$p < 0.01$; ****$p < 0.0001$

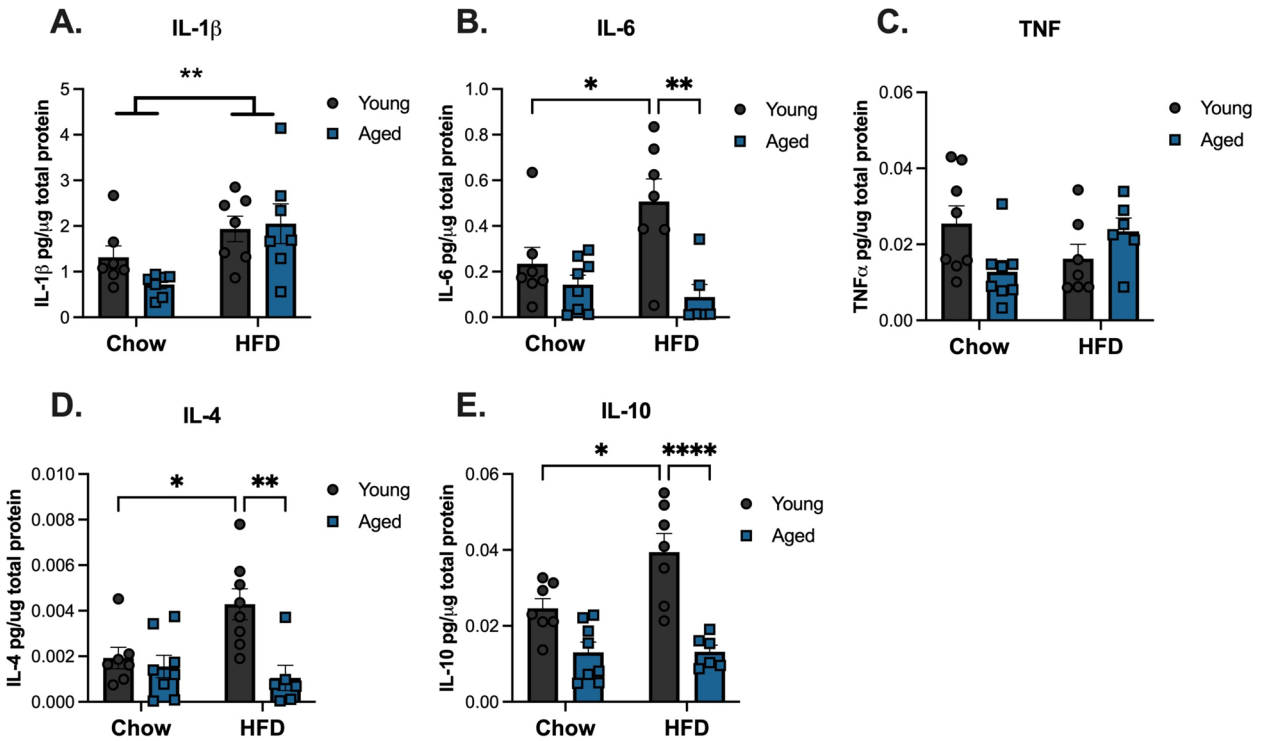

Fig. 9 Pro and anti-inflammatory markers in the distal colon of young and aged rats following long-term HFD consumption. Protein levels of (**A**) IL-1β, (**B**) IL-6, (**C**) TNF, (**D**) IL-4, and (**E**) IL-10 expressed as pg/g of total protein. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$

inflammatory markers in both the hippocampus and amygdala (Fig. 14D). On the other hand, Lactococcus, upregulated by HFD, positively correlated with all brain inflammatory markers (Fig. 14D).

Discussion

In this study we evaluated how long-term or short-term HFD consumption impacted memory function and anxiety-like behavior, central and peripheral inflammation, and gut microbiome profile in young adult and aged rats. Cognitive decline and affective disorders are a major concern for obese patients, and is observed in many obesity-associated comorbidities, such as type II diabetes [1, 45]. We found, consistent with other recent reports [10, 46–52], that cognitive impairments and increased anxiety-like behavior are present following long-term (3 months) HFD consumption in aged male rats. Critically, we show that these behavioral changes develop rapidly (within 3 days) after HFD onset and persist for months while on the diet. These data offer an important extension of our prior work [9, 10] by showing that rapid HFD-induced behavioral changes are not limited to episodic memory, but also extend to affective-like behavior and persist for months while the diet is being consumed. For both types of behavior, the rapid nature in which these changes emerge is striking and warrants further mechanistic investigation. Additionally, it should be noted that due to variations in macronutrients besides fat, we cannot rule out a role for changes in protein and/or carbohydrate content in the observed effects; the contribution of other macronutrients to the rapid neuroinflammatory response and cognitive and behavioral effects should be a focus of future studies.

HFD rapidly induces cognitive and behavioral changes

In the current study, consistent with other recent reports [46, 47, 49, 53], we found that long-term contextual and cued-fear memory impairments ensued following chronic (3 months) HFD consumption in aged male rats. Critically, we show that these impairments develop rapidly (within 3 days) after HFD onset and persist for months while maintained on the diet. These findings are consistent with our previous reports demonstrating long-term contextual and cued-fear memory deficits [9, 10], as well as impaired synaptic plasticity [54] after 3 days of HFD in aged, but not young adult male rats. While we did not observe memory deficits in young adult rats following either short- or long-term HFD consumption in the current study, HFD-induced spatial memory deficits have been reported in young rodents in the literature (reviewed by [55]. Additionally, we have previously reported contextual memory deficits in young adult

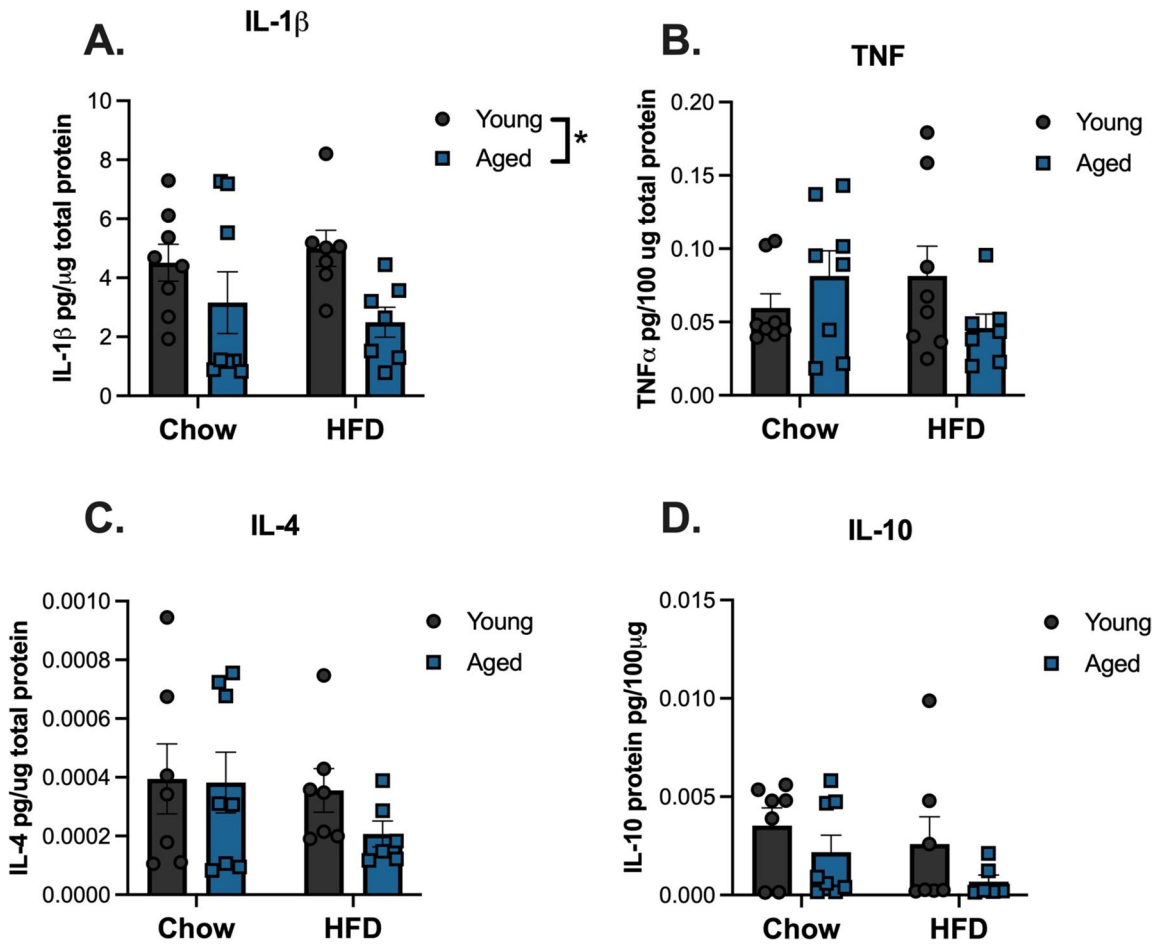

Fig. 10 Pro and anti-inflammatory markers in the ileum of young and aged rats following long-term HFD consumption. Protein levels of (**A**) IL-1β, (**B**) TNF, (**C**) IL-4, and (**D**) IL-10 expressed as pg/g of total protein. *$p < 0.05$

Wistar rats following 4–6 months of HFD consumption using a modified contextual pre-exposure fear-conditioning paradigm [40]. While differences between species, strain, and age of subjects, differences in diet compositions, and variations in behavioral procedures may contribute to the different results, this raises the need for future studies assessing additional cognitive domains following HFD.

We observed increased anxiety-like behavior in aged rats following either 3-months or 3-days of HFD; this was demonstrated by a reduction in the time spent in the center of the OFT chamber and an increase in time spent freezing compared to aged chow-fed rats. It is worth noting that we also observed a main effect of age to reduce anxiety-like behavior relative to young adult rats. This finding is consistent with several previous reports [56, 57], although others have shown aging to be anxiogenic in male rodents [46, 58, 59]. Even within studies, presence of anxiety-like behaviors can vary based on the behavioral task used. Although it is difficult to pinpoint exact reasons for the discrepancies between our findings and others, similar to the memory results it may relate to differences in subjects, diets, and exact behavioral parameters (time in chamber, lighting, habituation, order of tests), or the various combination of these factors.

Neuroimmune dysregulation occurs rapidly following HFD onset

Previous research demonstrated that HFD consumption and diet-induced obesity are associated with neuroimmune dysregulation, including in brain regions critical for long-term memory function and anxiety-like behavior [1]. Here, our data replicated these findings by demonstrating significant dysregulation of pro and anti-inflammatory cytokines in the hippocampus following long-term HFD consumption. Specifically, we report significant decreases in both pro and anti-inflammatory cytokines in the hippocampus of aged HFD-fed rats. It is important to note that even *decreases* in inflammatory cytokines leads to synaptic plasticity impairments

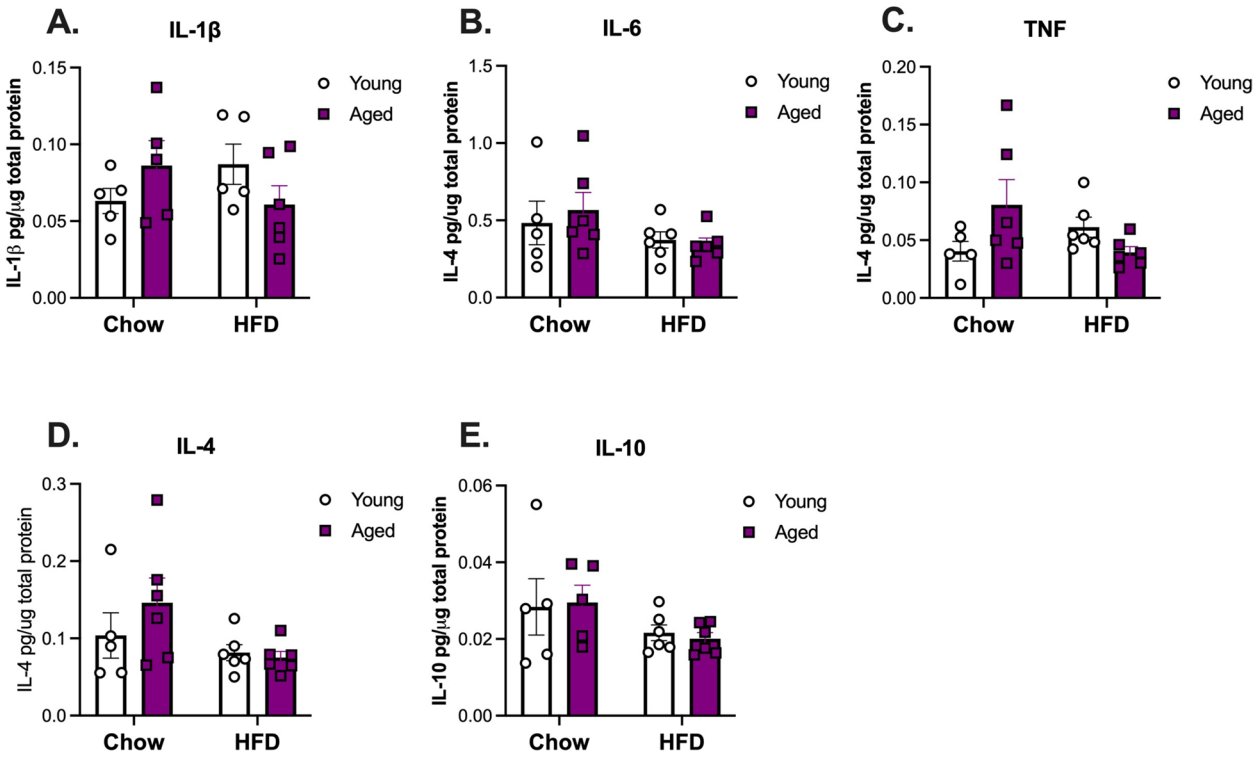

Fig. 11 Pro and anti-inflammatory markers in the distal colon of young and aged rats following short-term HFD consumption. Protein levels of (**A**) IL-1β, (**B**) IL-6, (**C**) TNF, (**D**) IL-4, and (**E**) IL-10 expressed as pg/g of total protein

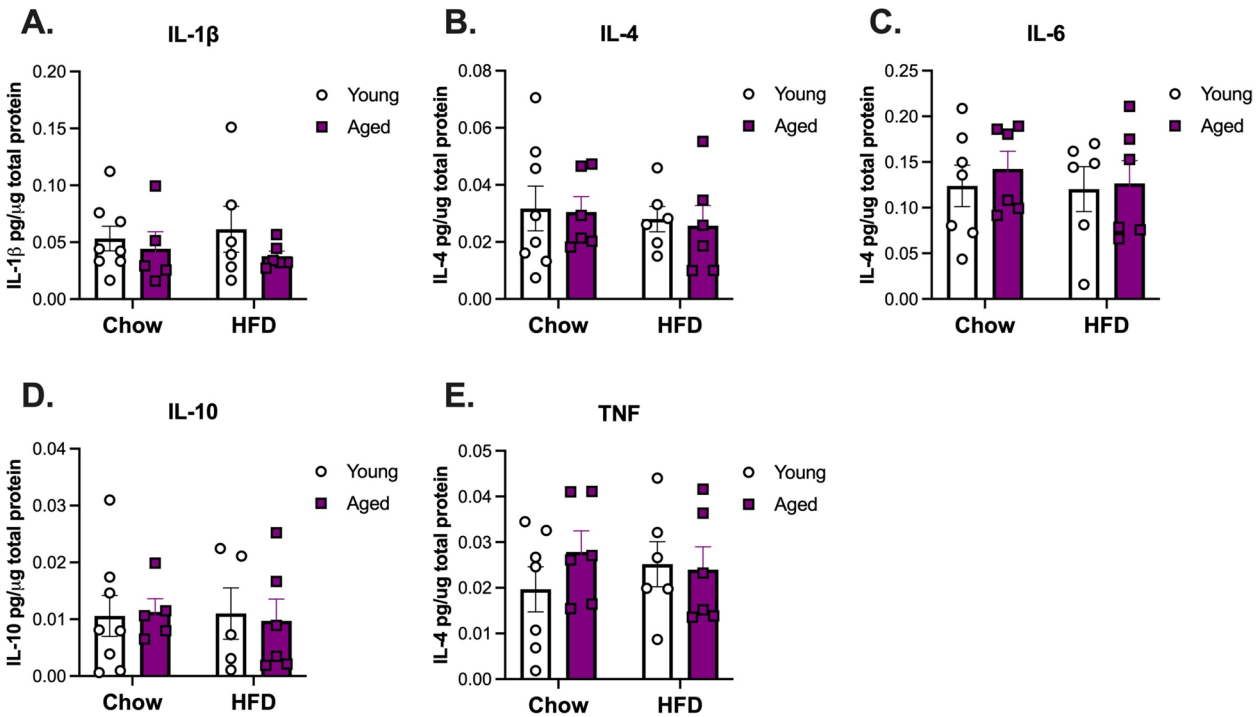

Fig. 12 Pro and anti-inflammatory markers in the ileum of young and aged rats following short-term HFD consumption. Protein levels of (**A**) IL-1β, (**B**) IL-6, (**C**) TNF, (**D**) IL-4, and (**E**) IL-10 expressed as pg/g of total protein

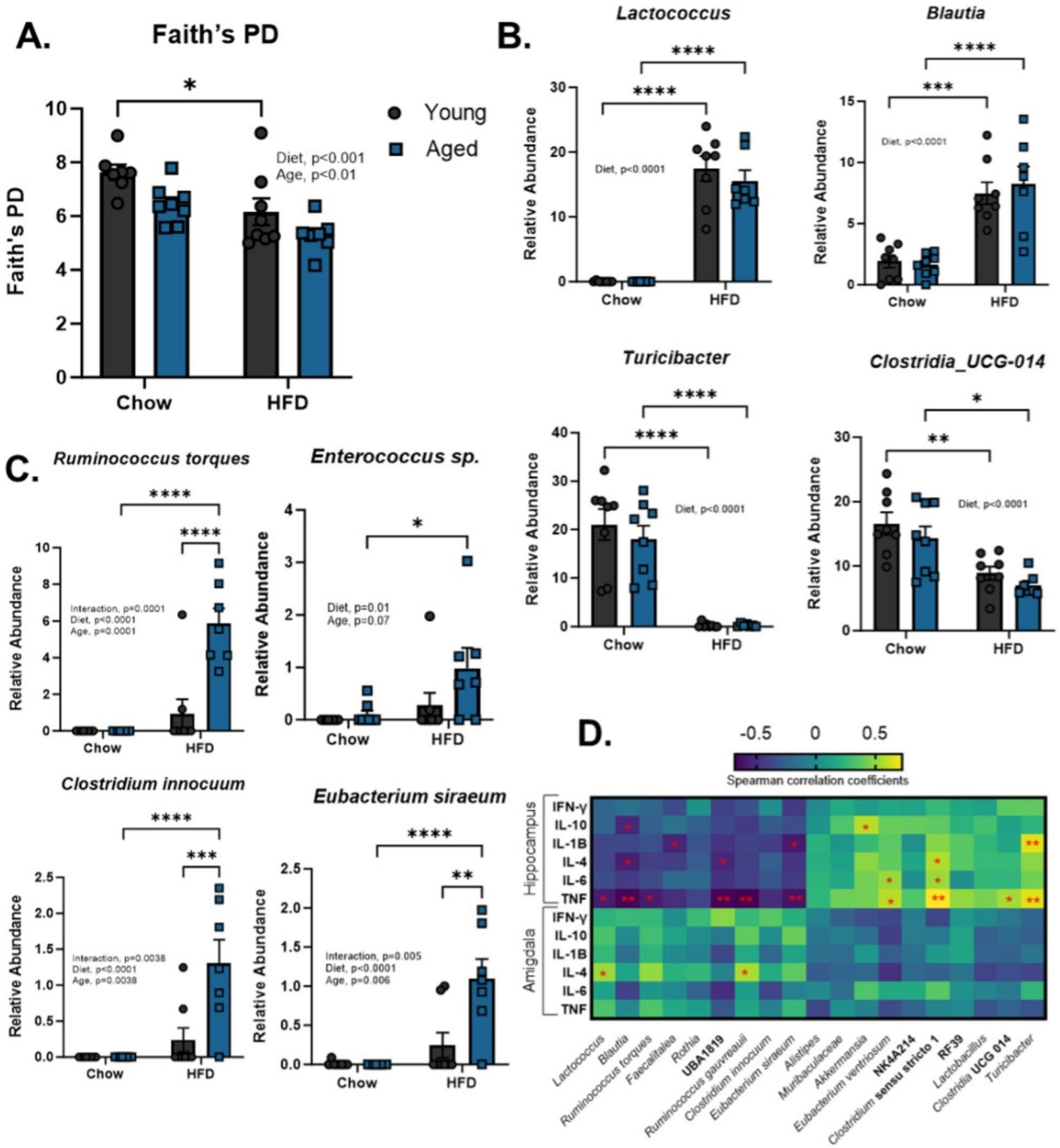

Fig. 13 Three months ingestion of high fat diet (HFD) robustly alters colon microbiome composition dependent of age. **A** Alpha-diversity indices as measured by Faith's PD. **B** Four top bacterial taxa (represented as % total bacteria) upregulated (*Lactococcus, Blautia*) and downregulated (*Turicibacter, Clostridia* UCG0-14) by HFD independent of age. **C** Two factor negative binomial (NEGBIN) reveals age x diet interactions for four bacterial taxa (adjusted $p < 0.05$). **D** Heatmap representing Spearman correlation coefficients comparing bacterial genera abundance vs. brain region specific protein levels in the aged group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

[20], suggesting cytokine dysregulation, in either direction, can cause cognitive deficits. Following short-term HFD consumption, we observed an elevation of IL-1β in the hippocampus, which replicated our previous work that implicated a causal role for this cytokine in mediating rapid HFD-induced late-phase LTP deficits and long-term memory impairments in aged rats [9, 54]. We extend these data by showing HFD rapidly alters other

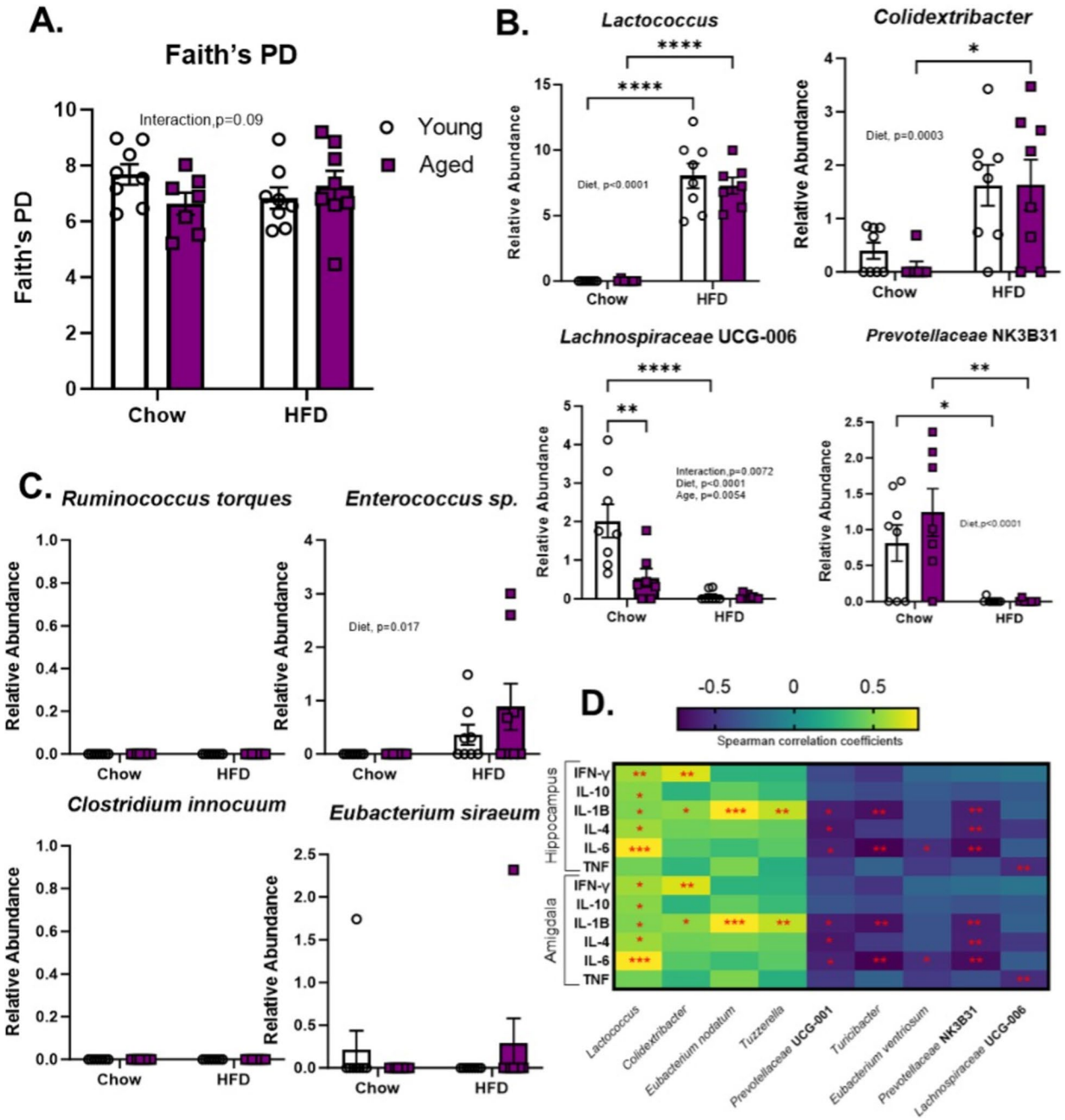

Fig. 14 Three days ingestion of high fat diet (HFD) moderately shifts colon microbiome composition independent of age. **A** Alpha-diversity indices as measured by Faith's PD. **B** Four top bacterial taxa (represented as % total bacteria) upregulated (*Lactococcus, Colidextribacter*) and downregulated (*Lachnospiraceae* UCG-006, *Prevotellaceae* NK3B31) by HFD independent of age. **C** Two factor negative binomial (NEGBIN) does not indicate a significant age x diet interactions for bacterial taxa. **D** Heatmap representing Spearman correlation coefficients comparing bacterial genera abundance vs. brain region protein levels in the aged group. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$

proinflammatory cytokines, including TNF, IL-6, and IFNγ, with IL-6 also being specifically upregulated in aged rats fed a HFD.

In the amygdala, we report increased proinflammatory cytokines as a function of age and long-term HFD consumption, particularly with IL-1β, TNF, and IFNγ proteins in aged HFD-fed rats. This predominantly proinflammatory phenotype in the aged amygdala following 3-month HFD is consistent with a deficit in memory function and an increase in anxiety-like

behavior [46, 47, 49–51]. Similar to the hippocampal data, we do see changes to amygdala cytokines that occur rapidly (within 3 days) of HFD consumption. Indeed, we observed a significant increase in IL-1β protein in aged rats that received a HFD, relative to young. However, this effect was not as robust as the hippocampal changes. These less robust changes in the amygdala could be due to a combination of two factors: differing kinetics of the inflammatory response between the amygdala and hippocampus, and less consistent tissue dissections as the amygdala lacks distinct anatomical landmarks, making fresh tissue dissections of the amygdala more challenging than the hippocampus. Nevertheless, diet- and age-associated cytokine alterations in the amygdala are consistent with our previous work [8, 9]. Taken together, these two experiments suggest that the rapid dysregulation of cytokines in the hippocampus and amygdala in aged HFD-fed rats persists for months upon continued consumption of a HFD.

Peripheral inflammation develops slowly following HFD onset

Because obesity is associated with widespread inflammation, we also investigated inflammatory signaling in adipose tissue and the gut. We saw a significant increase in IL-1β in visceral adipose tissue and the distal colon following long-term HFD consumption in both young and aged rats, which replicates prior work that demonstrated visceral adipose tissue and intestinal inflammation is a major component of diet-induced obesity [42–44, 60, 61].

Interestingly, young adult, but not aged, rats also exhibited an increase in anti-inflammatory cytokines, IL-4 and IL-10, in the distal colon, following long-term HFD. Strikingly, these data mirror the inflammatory profile in the brain, where we observed a skewed proinflammatory phenotype in aged rats fed a long-term HFD. Together, these data indicate a tightly regulated inflammatory response that strives to maintain homeostasis is lacking in aged rats. Consequentially, aged rats are more vulnerable to a proinflammatory phenotype in the gut and brain during diet-induced obesity, which is also consistent with the robust memory impairments observed in aged, but not young rats.

Importantly, cytokine dysregulation in adipose and gut tissue was not observed in either age group after short-term HFD consumption. These findings indicate that adipose and gut inflammation take longer to develop than neuroinflammation and provide strong support against their necessity for diet-induced memory or affective behavioral impairments. While this is the first study to demonstrate that diet-induced neuroinflammation in aging precedes gut and adipose tissue inflammation, the idea that neuroinflammation develops rapidly following HFD consumption is not novel, as this has been observed in the hypothalamus [62–64]. In fact, previous research demonstrated that hypothalamic inflammation preceded both systemic inflammation and significant weight gain following HFD consumption [64–68]. Here, we extend those data by showing a rapid inflammatory response in the aged hippocampus and amygdala that precedes several peripheral perturbations.

HFD rapidly induces changes in the gut microbiome

Examination of the gut microbiome revealed profound diet-induced changes to gut microbes following long term-HFD, with some alterations being strongly age-dependent. Indeed, HFD led to a striking increase in *Ruminococcus torques*, but only in aged rats. This taxa has been previously associated with inflammatory diseases such as inflammatory bowel disease [69] and increased by high-fat and high-sugar diets [70, 71]. Interestingly, *R. torques* also has mucolytic (mucus degrading) properties and has been strongly implicated in the pathogenesis of colorectal cancer in humans [72].

In contrast to long-term HFD, short-term HFD-induced changes to the microbiome occurred largely independent of age. For example, *Lactococcus* sp. and *Enterococcus* sp. expanded in both young and aged HFD-fed rats. We did observe some age-associated bacteria that were more sensitive to HFD. This included *Lachnospiraceae* UCG-006, which was lower in aged rats and was significantly downregulated by HFD. Interestingly, we found *Lachnospiraceae* UCG-006- a known producer of immunomodulatory short chain fatty acids (SCFA)- was negatively associated with TNF concentration in both the hippocampus and the amygdala in aged rats. These data indicate that early microbiome shifts induced by HFD, may play a role in neuroinflammatory processes and behavioral abnormalities linked to both HFD and aging, but these findings will need to be confirmed with future mechanistic studies.

Conclusions

Taken together, our data demonstrated that long-term (3 months) HFD consumption resulted in memory impairments and increased anxiety-like behavior in aged, but not young rats, which could not solely be explained by HFD-evoked dysregulation of brain cytokines or alterations in fasting glucose and insulin, as these were observed in both age groups. Increased expression of anti-inflammatory cytokines in the hippocampus and distal colon, and decreased expression of inflammatory microbes in the gut microbiome of young adult rats may explain this discrepancy in behavioral outcomes. In contrast, short-term (3 days) HFD, which caused these same behavioral deficits in

aged rats, was accompanied by robust dysregulation of brain cytokines selectively in aged rats, and occurred in the absence of changes to fasting metabolic markers or peripheral inflammation in adipose tissue or colon. Although a few shifts in gut microbiome composition did occur with HFD rapidly, they occurred equally in both young and aged rats, indicating that other age-related mechanisms may underlie the enhanced and rapid neuroinflammatory sensitivity to diet. Overall, these data suggest that HFD-evoked neuroinflammation, memory impairments, and anxiety-like behavior in aging develop quicker and separately from the peripheral hallmarks of diet-induced obesity.

Abbreviations

HFD	High fat diet
OFT	Open field test
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
TNF	Tumor necrosis factor
IL-10	Interleukin 10
IL-4	Interleukin 4
IFNγ	Interferon gamma

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12979-024-00496-3.

Supplementary Material 1: Supplementary Figure 1. Three-month ingestion of high fat diet (HFD) robustly alters colon microbiome composition dependent of age. (A) Alpha-diversity as measured by Shannon Index. Beta-diversity represented by (B) LDA scores for the effect of HFD feeding on bacterial taxa, and (C) Heat trees depicting bacterial taxa differences between HFD vs. chow within age groups (left: Young; right: Aged).

Supplementary Material 2: Supplementary Figure 2. Three-day ingestion of high fat diet (HFD) moderately shifts colon microbiome composition independent of age. (A) Alpha-diversity indices as measured by Shannon Index. Beta-diversity represented by (B) LDA score for the effect of HFD feeding on bacterial taxa, and (C) Heat trees depicting bacterial taxa differences between HFD vs. chow within age groups (left: Young; right: Aged).

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Authors' contributions
MJB & SMM: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. MECS, AS, & RHM: Methodology, Investigation, Writing – review & editing. BGO, SMA, NM, BDA, JAB, MNB, & JWD: Investigation, Writing – review & editing. JMA & RMB: Conceptualization, Supervision, Investigation, Funding acquisition, Writing – review & editing. All authors have approved the final version of this manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable

Competing interests
The authors declare no competing interests.

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