Supplementary Information (SI)

Brain sex-dependent alterations in anterior cortex after prolonged high fat diet exposure:

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Supplementary Figure 1: BMI of male and female mice after 7 and 12 weeks of high fat diet consumption

(a) BMI in males and females at 7 weeks of diet. (b) BMI in males and females at 12 weeks of diet. Data were analyzed using Two-Way ANOVA including sex and diet as variable and were expressed as mean ±SD. * p≤0.05; *** p≤0.001
Supplementary Figure 2: Biochemical parameters of females and males fed with high fat diets for 14 and 33 weeks

45HFD (left panel) and 60HFD (right panel) vs STD male and female at 14 (14w) and 33 weeks (33w); (a) High Dense Lipoprotein (HDL); (b) Low Dense Lipoproteins (LDL); (c) Aspartate transaminase enzyme (AST); (d) Alanine transaminase enzyme (ALT); (e) Albumin (Alb). Data were analyzed using Three-Way ANOVA followed by Tukey’s post hoc test for multiple comparison using sex and diet as covariate and are expressed as mean ± SD.

$ significance between HFD males and HFD females at 14 weeks; # significance between HFD males and HFD females at 33 weeks. * p≤0.05; *** p≤0.001
Supplementary Figure 3: GO and KEGG analysis in 60HFD and 45HFD male mice
(a) Differentially expressed genes (DEGs) derived from GO analysis in 60HFD male anterior cortex compared to STD control diet. (b) DEGs derived from KEGG analysis in 60HFD male anterior cortex compared to STD control diet. (c) Differentially expressed genes (DEGs) derived from GO analysis in 45HFD male anterior cortex compared to STD control diet. (d) DEGs derived from KEGG analysis in 45HFD male anterior cortex compared to STD control diet. Data were analyzed using One-Way ANOVA with the cutoff of p-value≤0.05 and fold change≥2. The length of the bar represents the significance of that specific gene-set or term. Significance of the specific gene-set is represented by the brightness of the color.
**Supplementary Figure 4: GO and KEGG analysis in 45HFD female mice**

(a) Differentially expressed genes (DEGs) derived from GO analysis in 60HFD female anterior cortex compared to STD control diet. (b) DEGs derived from KEGG analysis in 60HFD female anterior cortex compared to STD control diet. (c) Differentially expressed genes (DEGs) derived from GO analysis in 45HFD female anterior cortex compared to STD control diet. (d) DEGs derived from KEGG analysis in 45HFD female anterior cortex compared to STD control diet. Data were analyzed using One-Way ANOVA with the cutoff of p-value≤0.05 and fold change≥2. The length of the bar represents the significance of that specific gene-set or term. Significance of the specific gene-set is represented by the brightness of the color.
Supplementary Figure 5. Western blot quantification of Glial Fibrillary Acidic Protein (GFAP) protein in male and female anterior cortex at 35 weeks of diet. Data obtained from technical replicate analyses, normalized on total proteins, were analyzed using Kruskal–Wallis test followed by Dunn's post hoc test and are expressed as mean ± S.E.M. vs STD.
Supplementary Figure 6: Ingenuity Pathway Analysis (IPA) results in Anterior Cortex. The most significantly altered pathways common to males (blue bars) and females (red bars) in HFD60 vs CTRL in the canonical pathway analysis are presented from the more to the less significant. X-axis: \(-\log (p\text{-value})\). The dotted line represents the \(p\text{-value} = 0.05\). IPA's z-scores are indicated at the end of each bar. N/A means that IPA is not able to assign an activity pattern to a given pathway. Arrows on the left indicate the pathways, which had a positive z-score.
Most significantly altered pathways in female cerebellum (IPA)

Supplementary Figure 7: Ingenuity Pathway Analysis (IPA) results in cerebellum. The most significantly altered pathways in female cerebellum in HFD45 vs STD (a) and in HFD60 vs STD (b) in the canonical pathway analysis are presented from the more to the less significant. X-axis: -log (p-value). The dotted line corresponds to 1.3 (-log 0.05). IPA’s z-scores are indicated at the end of each bar. N/A: IPA is not able to assign an activity pattern to a pathway.
Supplementary Figure 8. Venn diagram analyses
a. Principal Component analysis of males and females DEGs in Cb and ACX. DEGs in Cb and ACX show a high degree of segregation indicating specific gene expression patterns in the two brain regions. b-c Differentially Expressed Genes (DEGs) common to Cb and ACX in animals fed on 45HFD (516 (9.9%), b) and 60HFD (442 (9.1%), c). d-e GO-Terms significantly enriched in DEGs common to Cb and ACX of Males and Females fed on 45HFD (516 (9.9%, d) and of Males and Females fed on 60HFD (442 (9.1%); e. f. Venn diagram representation of DEGs of the ACX common to males and females fed on 60HFD (785 (28.1%) and GO-Terms enrichment. g-h Venn diagram representation of DEGs of the Cb ACX common to males and females fed on 45HFD (g) and on 60HFD (h). The GO bars are sorted by p-Value rankings represented by the bar length and colors indicating significant enrichments below the threshold of 0.05.
Supplementary TABLE 1 High Fat Diet and Standard Diet Fat composition

| FAT    | %   | Saturated | Monounsaturated | Polyunsaturated | Main ingredient |
|--------|-----|-----------|-----------------|-----------------|----------------|
| STD    | 10% | 23%       | 30%             | 47%             | WHEAT          |
| 45HFD  | 45% | 31%       | 36%             | 33%             | LARD           |
| 60HFD  | 60% | 32%       | 36%             | 32%             | LARD           |

Supplementary TABLE 2 Sample size for each experimental task

| Task   | Time (weeks) | BMI | PET § | GTT | Post mortem* | Hemato# |
|--------|--------------|-----|-------|-----|--------------|---------|
|        |              |     | FDG PET | VC701 PET |            |         |
| M      |              | 14  | 33     | 7    | 12 | 31         | 31      | 33 | 14 | 33 |
| 60% HFD | 5  | 5     | 8    | 8    | 3  | 6          | 6       | 3  | 5  | 10  | 5 |
| 45% HFD | 5  | 5     | 8    | 8    | 4  | 6          | 6       | 3  | 5  | 10  | 5 |
| STD    | 5  | 5     | 8    | 7    | 4  | 6          | 6       | 3  | 5  | 10  | 5 |
| F      |              | 5  | 5     | 8    | 8    | 4  | 6          | 6       | 3  | 5  | 10  | 5 |
| 60% HFD | 5  | 5     | 8    | 8    | 4  | 6          | 6       | 3  | 5  | 10  | 5 |
| 45% HFD | 5  | 5     | 8    | 8    | 4  | 6          | 6       | 3  | 5  | 10  | 5 |
| STD    | 5  | 5     | 8    | 8    | 4  | 6          | 6       | 3  | 5  | 10  | 5 |

§ PET studies with FDG and VC701 were performed starting during week 7, 12 and 31 on different days with a minimum distance of 48 hours. For each PET section, a maximum of 12 mice for FDG and 9 for VC701 were acquired with the same radiopharmaceutical preparation. Male and Female started the diet and were processed in separate days. #Hematochemistry 14 w: 5 mice from PET group (group 1) and liver group (group 2) were sacrificed and used also for hematochemistry analysis. * Female STD group for western blot, n=4; 1 Male of 45HFD, n=4: spontaneously died.
### Supplementary TABLE 3: Spearman r and p values of PET [¹⁸F]FDG correlations analysis for males (A) and females (B)

**A**

|                | Glycaema | BMI   | Tot. Chol | HDL  | LDL  | AST   | ALT   | Albumin |
|----------------|----------|-------|-----------|------|------|-------|-------|---------|
| 31 weeks - Males |          |       |           |      |      |       |       |         |
| Olfactory bulb   | 0.47     | 0.179 | 0.71      | 0.027| 0.79 | 0.009 | 0.81  | 0.006   |
| Anterior cortex  | 0.37     | 0.296 | 0.52      | 0.133| 0.66 | 0.044 | 0.55  | 0.101   |
| Whole cortex     | 0.15     | 0.674 | 0.45      | 0.192| 0.66 | 0.043 | 0.62  | 0.061   |
| Striatum         | 0.33     | 0.353 | 0.38      | 0.282| 0.61 | 0.067 | 0.51  | 0.134   |
| Hippocampus      | -0.22    | 0.542 | -0.01     | 0.980| 0.22 | 0.542 | 0.18  | 0.609   |
| Hypothalamus     | -0.01    | 0.994 | 0.08      | 0.831| 0.29 | 0.420 | 0.19  | 0.602   |
| Thalamus         | -0.22    | 0.542 | -0.27     | 0.453| -0.15| 0.688 | -0.17 | 0.639   |
| Brainstem        | -0.33    | 0.349 | -0.31     | 0.388| -0.40| 0.254 | -0.39 | 0.263   |
| Cerebellum       | -0.51    | 0.139 | -0.72     | 0.024| -0.89| 0.001 | -0.89 | 0.001   |
| SUV whole brain  | -0.71    | 0.027 | -0.54     | 0.114| -0.62| 0.060 | -0.57 | 0.093   |

**B**

|                | Glycaema | BMI   | Tot. Chol | HDL  | LDL  | AST   | ALT   | Albumin |
|----------------|----------|-------|-----------|------|------|-------|-------|---------|
| 31 weeks - Females |          |       |           |      |      |       |       |         |
| Olfactory bulb   | 0.39     | 0.257 | 0.68      | 0.025| 0.25 | 0.446 | 0.43  | 0.184   |
| Anterior cortex  | 0.59     | 0.077 | 0.10      | 0.760| 0.31 | 0.350 | 0.21  | 0.543   |
| Whole cortex     | 0.13     | 0.727 | -0.05     | 0.895| 0.12 | 0.715 | 0.23  | 0.495   |
| Striatum         | 0.28     | 0.431 | 0.34      | 0.297| 0.42 | 0.197 | 0.56  | 0.075   |
| Hippocampus      | 0.09     | 0.803 | -0.02     | 0.949| 0.04 | 0.909 | 0.34  | 0.308   |
| Hypothalamus     | 0.17     | 0.631 | 0.53      | 0.099| 0.13 | 0.699 | 0.31  | 0.351   |
| Thalamus         | 0.13     | 0.720 | 0.23      | 0.498| 0.24 | 0.466 | 0.49  | 0.132   |
| Brainstem        | 0.06     | 0.872 | 0.44      | 0.177| -0.03| 0.921 | 0.02  | 0.953   |
| Cerebellum       | -0.41    | 0.235 | -0.54     | 0.090| -0.27| 0.417 | -0.46 | 0.158   |
| SUV whole brain  | -0.15    | 0.684 | -0.03     | 0.930| 0.19 | 0.570 | 0.28  | 0.405   |
### Supplementary TABLE 4

Spearman r and p values of PET [¹⁸F]VC701 correlations analysis for males (A) and females (B)

| A | Glycaemia | BMI | Tot. Chol | HDL | LDL | AST | ALT | Albumin |
|---|-----------|-----|-----------|-----|-----|-----|-----|---------|
| 31 weeks - Males | r | p | r | p | r | p | r | p | r | p | r | p |
| Olfactory bulb | 0.43 | 0.299 | 0.47 | 0.213 | 0.55 | 0.171 | 0.24 | 0.582 | **0.78** | **0.026** | 0.64 | 0.096 | 0.71 | 0.058 | 0.76 | 0.071 |
| Anterior cortex | 0.38 | 0.360 | 0.60 | 0.097 | **0.79** | **0.028** | 0.40 | 0.327 | **0.80** | **0.023** | 0.62 | 0.115 | **0.74** | **0.046** | 0.76 | 0.071 |
| Whole cortex | 0.29 | 0.501 | 0.63 | 0.076 | **0.81** | **0.022** | 0.43 | 0.299 | 0.71 | 0.056 | 0.52 | 0.197 | 0.67 | 0.083 | 0.63 | 0.143 |
| Striatum | 0.29 | 0.501 | 0.57 | 0.121 | **0.74** | **0.046** | 0.33 | 0.428 | **0.88** | **0.007** | 0.50 | 0.216 | 0.62 | 0.115 | 0.76 | 0.071 |
| Hippocampus | 0.38 | 0.360 | 0.60 | 0.097 | **0.79** | **0.028** | 0.40 | 0.327 | **0.80** | **0.023** | 0.62 | 0.115 | **0.74** | **0.046** | 0.76 | 0.071 |
| Hypothalamus | 0.38 | 0.360 | 0.60 | 0.097 | **0.79** | **0.028** | 0.40 | 0.327 | **0.80** | **0.023** | 0.62 | 0.115 | **0.74** | **0.046** | 0.76 | 0.071 |
| Thalamus | 0.19 | 0.665 | 0.53 | 0.148 | 0.69 | 0.069 | 0.31 | 0.462 | **0.83** | **0.014** | 0.43 | 0.299 | 0.55 | 0.171 | 0.76 | 0.071 |
| Brainstem | 0.29 | 0.501 | 0.48 | 0.194 | **0.74** | **0.046** | 0.38 | 0.360 | **0.75** | **0.040** | 0.55 | 0.171 | 0.67 | 0.083 | 0.76 | 0.071 |
| Cerebellum | 0.48 | 0.243 | 0.67 | 0.059 | **0.83** | **0.015** | 0.45 | 0.268 | 0.63 | 0.106 | 0.67 | 0.083 | **0.79** | **0.028** | 0.76 | 0.071 |

| B | Glycaemia | BMI | Tot. Chol | HDL | LDL | AST | ALT | Albumin |
|---|-----------|-----|-----------|-----|-----|-----|-----|---------|
| 31 weeks - Females | r | p | r | p | r | p | r | p | r | p | r | p |
| Olfactory bulb | 0.48 | 0.243 | 0.08 | 0.854 | 0.05 | 0.912 | 0.00 | >0.999 | 0.19 | 0.665 | 0.33 | 0.428 | 0.57 | 0.151 | 0.13 | 0.857 |
| Anterior cortex | 0.40 | 0.327 | 0.00 | >0.999 | -0.07 | 0.880 | -0.24 | 0.582 | 0.19 | 0.665 | 0.07 | 0.882 | 0.45 | 0.268 | -0.13 | 0.857 |
| Whole cortex | 0.41 | 0.314 | 0.01 | 0.987 | 0.02 | 0.973 | -0.05 | 0.916 | 0.24 | 0.564 | 0.26 | 0.523 | 0.54 | 0.177 | 0.06 | 0.929 |
| Striatum | 0.44 | 0.273 | 0.03 | 0.942 | -0.06 | 0.886 | -0.19 | 0.647 | 0.14 | 0.736 | 0.14 | 0.736 | 0.49 | 0.222 | -0.06 | 0.929 |
| Hippocampus | 0.45 | 0.268 | 0.08 | 0.854 | -0.05 | 0.912 | -0.12 | 0.793 | 0.02 | 0.977 | 0.29 | 0.501 | 0.50 | 0.216 | 0.00 | >0.999 |
| Hypothalamus | 0.44 | 0.273 | 0.04 | 0.924 | 0.03 | 0.957 | 0.00 | >0.999 | 0.19 | 0.665 | 0.33 | 0.428 | 0.57 | 0.151 | 0.13 | 0.857 |
| Thalamus | 0.40 | 0.327 | -0.02 | 0.973 | -0.10 | 0.810 | -0.14 | 0.752 | 0.10 | 0.840 | 0.21 | 0.619 | 0.52 | 0.197 | 0.00 | >0.999 |
| Brainstem | 0.45 | 0.268 | 0.08 | 0.854 | -0.05 | 0.912 | -0.12 | 0.793 | 0.02 | 0.977 | 0.29 | 0.501 | 0.50 | 0.216 | 0.00 | >0.999 |
| Cerebellum | 0.52 | 0.197 | 0.15 | 0.700 | 0.00 | >0.999 | -0.12 | 0.793 | 0.02 | 0.977 | 0.29 | 0.501 | 0.50 | 0.216 | 0.00 | >0.999 |