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Cognitive Performance during the Development of Diabetes in the Zucker Diabetic Fatty Rat

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Supplementary

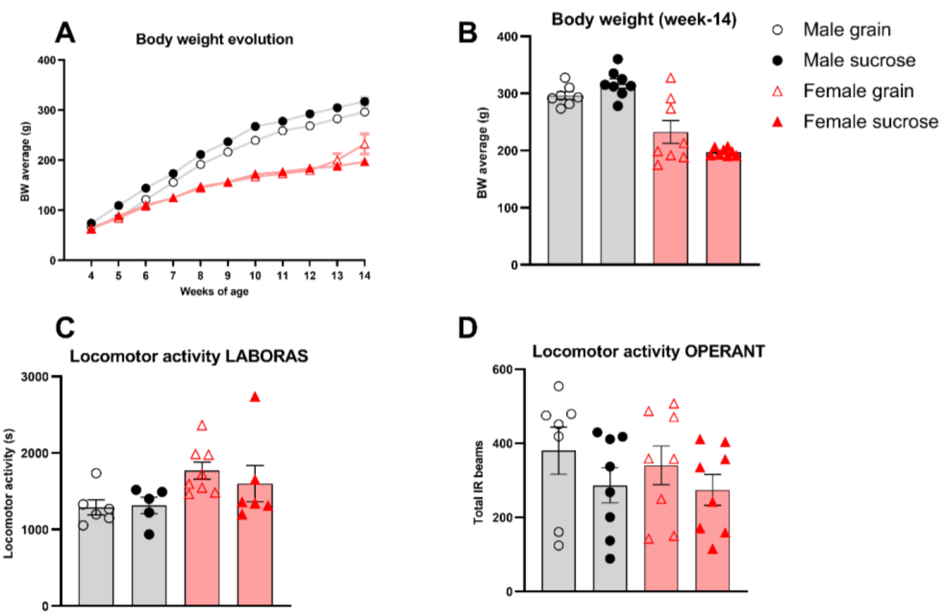

Figure S1. Body weight development and locomotor activity of male and female LE-sucrose and LE-grain rats. (A) Body weight evolution between weeks 4 and 14 of age. (B) Body weight comparison at week 14. (C) Values represent the mean of 5 sessions of 48h each in the Laboras homecage. (D) Locomotor activity during the visual discrimination (VD) stage. Values represent the group mean (±SEM). We observed a main effect of feeding regime ($F_{(1, 27)\ \text{feeding regime}} = 5.990$, p=0.021) and an interaction between sex and feeding regime ($F_{(1, 27)\ \text{sex x feeding regime}} = 9.491$, p=0.0047) during development ($F_{(10, 270)\ \text{sex x feeding regime x weeks}} = 1.983$, p=0.035). This indicated that LE-sucrose males reached a higher body weight compared to LE-grain males, but no main difference in body weight levels occurred between females. A significant effect of sex on locomotor activity in the Laboras cage was found, with females being more active than males ($F_{\text{feeding regime}(1,21)}=0.2327$, p=n.s., $F_{\text{sex}(1,21)}=6.466$, $p<0.05$).

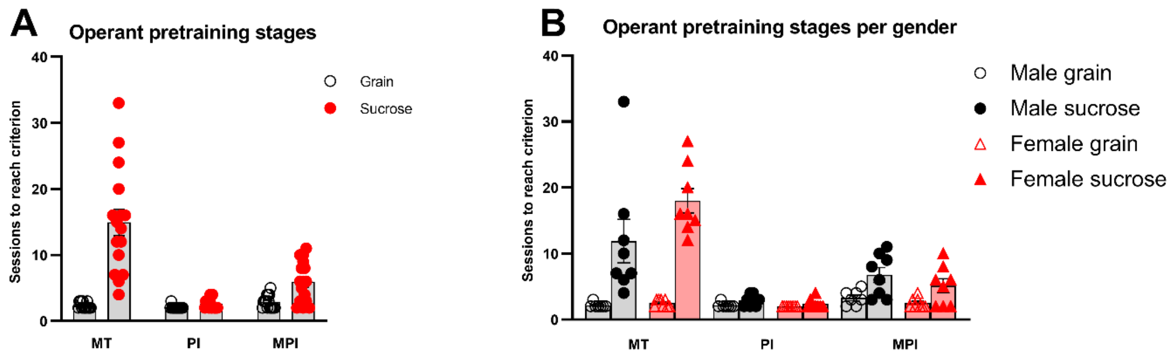

Figure S2. Number of sessions required for male and female LE-sucrose and LE-grain rats to reach criterion during the pretraining stages of the visual discrimination test. (A) Number of sessions required to reach criterion during the pretraining stages for LE rats receiving either grain or sucrose and (B) the same results specified per gender. Values represent the group mean (±SEM). LE-sucrose rats needed more sessions to reach criterion in all training stages: MT ($F_{\text{feeding regime}(1,\ 27)}$=41.30, p<0.0001), PI ($F_{\text{feeding regime}(1,\ 27)}$=6.652 p<0.05) and MPI ($F_{\text{feeding regime}(1,\ 27)}$= 3.26, p<0.01) (B). No significant effects of sex were observed. AS = autoshaping; MT = must touch; PI = punish incorrect; MPI = moving punish incorrect.

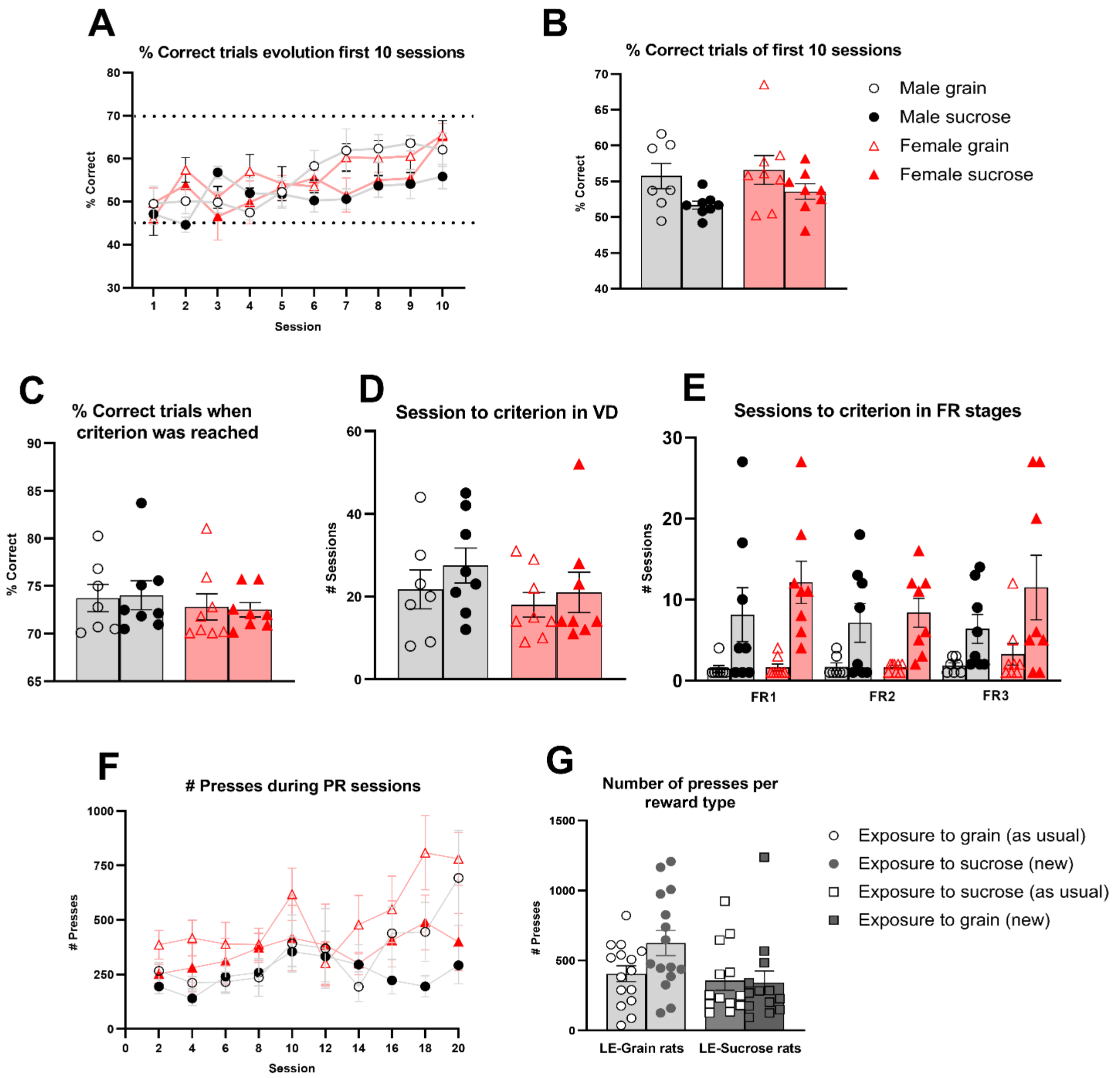

Figure S3. Results of the visual discrimination task, fixed ratio and progressive ratio tasks in male and female LE-sucrose and LE-grain rats. (A) Percentage of correct trials evolution during the 10 first sessions of the visual discrimination (VD) task. (B) Average percentage of correct responding during the first 10 sessions of the VD task. (C) Average percentage of 3 consecutive trials when criterion was reached. (D) Number of sessions required to reach criterion during the VD task. (E) Number of sessions required to reach criterion in different Fixed Ratio (FR) stages. (F) Average number of correct presses during the 20 progressive ratio (PR) sessions. (G) Effects of the reward type on the motivation to press for either sucrose or grain pellets. Values represent the group mean (±SEM). Mixed-effects model analysis across the first 10 visual discrimination sessions (A-B) showed that LE-sucrose rats had a lower percentage of correct responses compared to LE-grain rats ($F_{feeding\ regime(1,27)}$= 5.93, $p<0.05$). No significant effects were observed between grain and sucrose LE rats, once the VD criterion of three sessions on >70% was reached (C), nor did the number of sessions to obtain the VD criteria was different between groups (D). The number of sessions required to reach criteria during all FR tasks differed in feeding regime but not in sex: FR1 ($F_{sex(1,27)}$=0.89, p=n.s.; $F_{feeding\ regime(1,31)}$=15.10, $p<0.001$); FR2 ($F_{sex(1,27)}$=0.13, p=n.s.; $F_{feeding\ regime(1,27)}$=15.18, $p<0.001$); FR5 ($F_{sex(1,31)}$=1.89, p=n.s.; $F_{feeding\ regime(1,27)}$=7.26, $p<0.05$). In the progressive ratio stage, mixed-effects model analysis across the 20 sessions has been investigated. Only a significant effect of sex was found in which females performed more correct presses, but no difference in feeding regime was observed ($F_{sex(1,25)}$=7.03, $p<0.05$; $F_{feeding\ regime(1,25)}$=3.29, p=n.s.). However, when the effect of reward type on motivation was assessed, where the 3 sessions of the 20 PR sessions (16, 18, 20), were compared to sessions (15, 17, 19), with either sucrose or grain as the new reward type; the average number of presses for the sucrose reward type during these sessions almost reached significance indicating an overall higher motivation for sucrose rewards ($F_{reward\ type(1,26)}$=4.201, $p=0.051$).

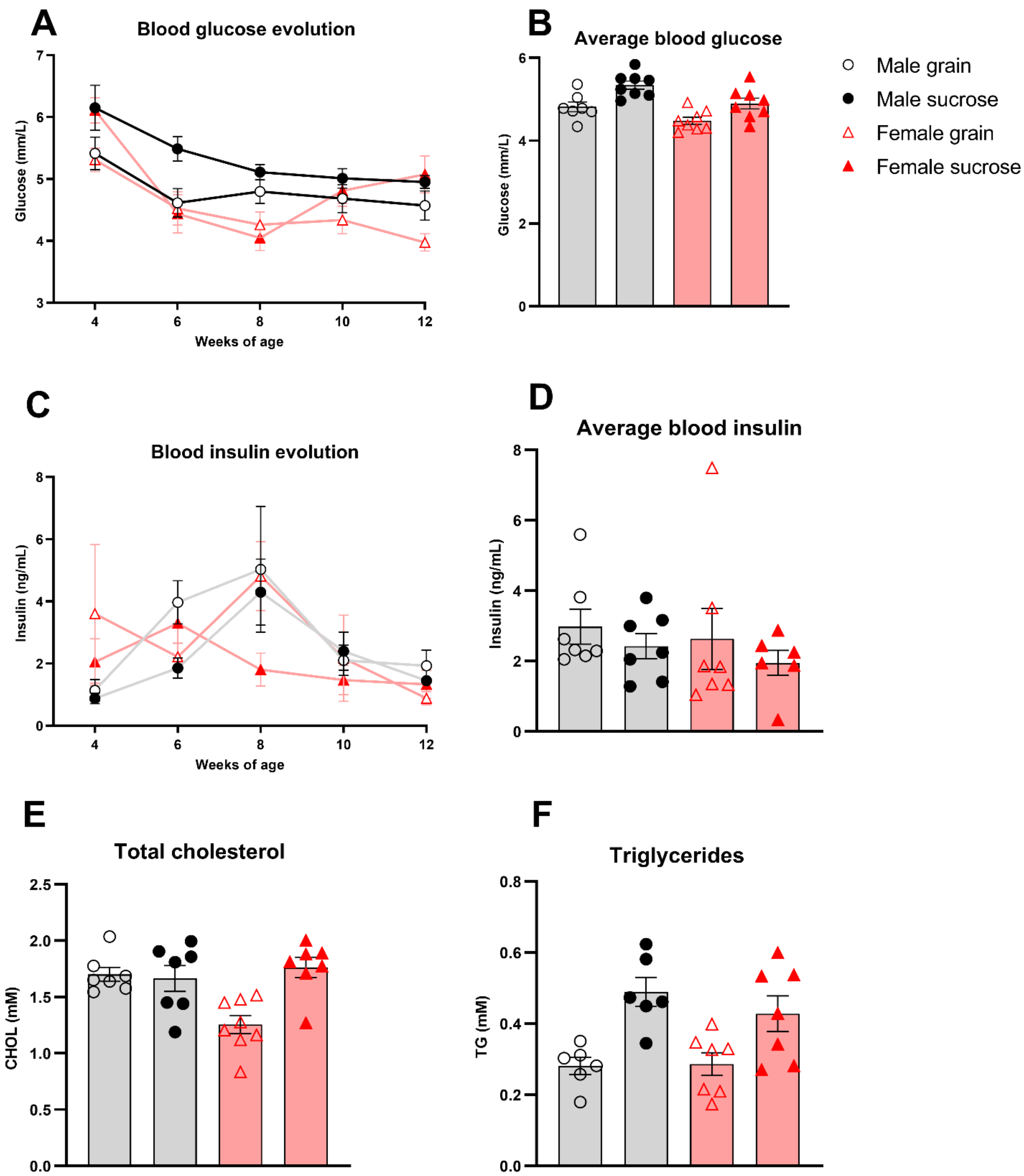

Figure S4. Blood glucose, insulin and lipid levels in male and female LE-sucrose and LE-grain rats. (A, C) Fasted glucose and insulin levels over time at weeks 4, 6, 8, 10 and 12. (B, D) Average of fasted glucose and insulin levels of weeks 4, 6, 8, 10 and 12. (E) Average total cholesterol levels at week 12. (F) Average total triglyceride levels at week 12. Values represent the mean (±SEM). LE-sucrose rats compared to LE-grain rats presented significantly higher levels of glucose (Glucose: $F_{feeding\ regime(1,27)}$= 17.96, p<0.001; $F_{sex(1,27)}$=12.35, p<0.01) but not of insulin ($F_{feeding\ regime(1,21)}$=0.06, p=n.s., $F_{sex(1,21)}$=1.47, p=n.s.). Compared to LE-grain, LE-sucrose rats presented significant higher levels of triglycerides ($F_{feeding\ regime(1,22)}$= 20.06, p<0.001; $F_{sex(1,22)}$= 0.52, p=n.s.) and cholesterol ($F_{feeding\ regime\ (1,25)}$=7.03, p<0.05; $F_{sex\ (1,25)}$=3.82, p=n.s.). Furthermore, a significant interaction effect between sex and feeding regime was found for total cholesterol ($F_{(1,\ 25)}$= 9.453, p=0.005). Subsequent multiple comparison analyses indicated that female LE sucrose rats showed higher levels of cholesterol compared to female LE grain rats (p<0.05) while no difference between the male groups was observed. .

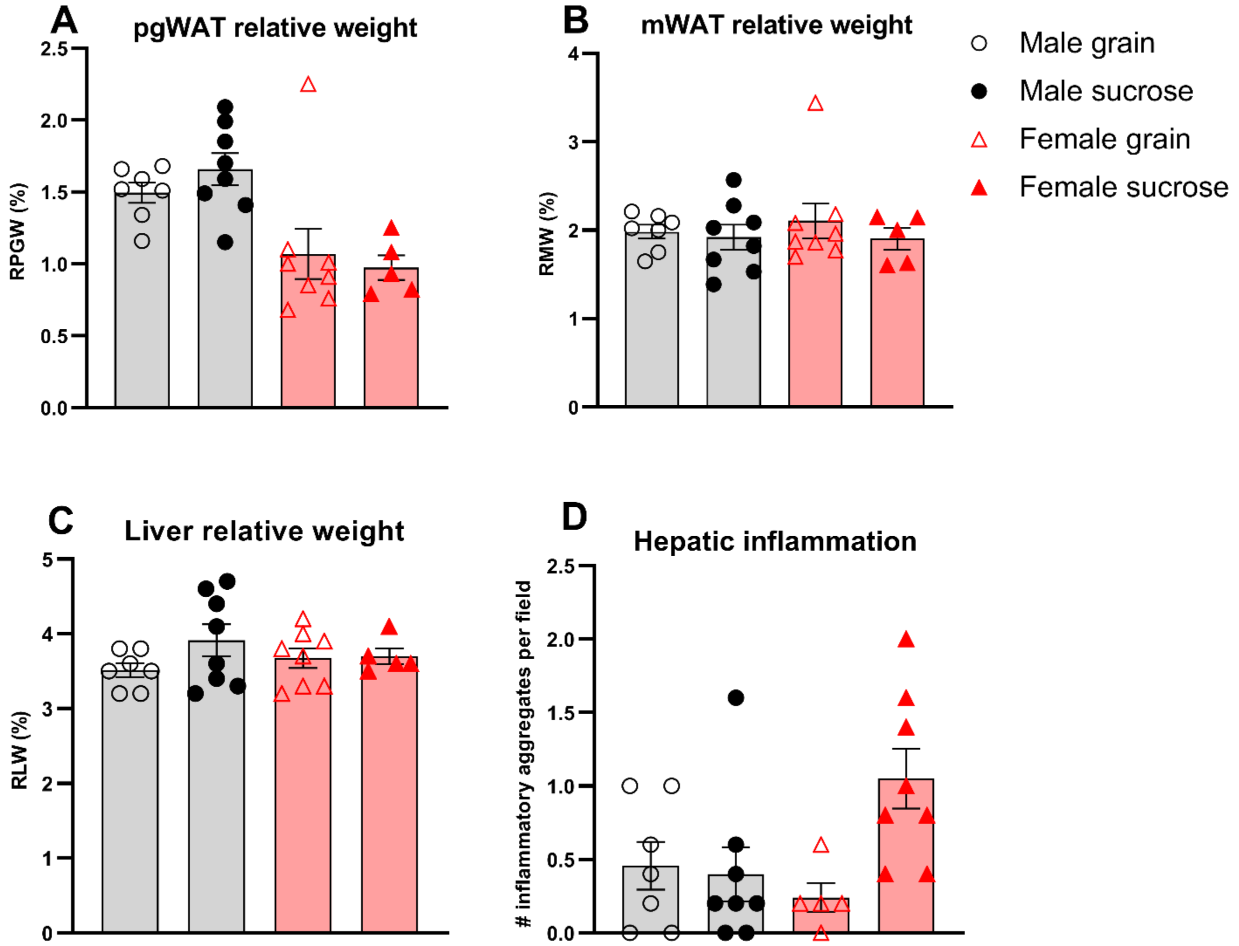

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Figure S5. Average relative weights of perigonadal and mesenteric white adipose tissues and liver histopathology in male and female LE-sucrose and LE-grain rats. (A, B) Average relative weights of perigonadal (pgWAT) and mesenteric (mWAT) white adipose tissues. (C) Relative liver weights. (D) Average of observed inflammatory aggregates within the liver tissue. Values represent the group mean (±SEM). No significant changes were found in relative pgWAT and mWAT in LE-sucrose rats compared LE-grain rats ($F_{feeding\ regime(1,24)}=0.06$, p=n.s.; $F_{feeding\ regime(1,24)}=0.73$, p=n.s.), respectively. No difference in relative liver weights was observed either ($F_{feeding\ regime(1,24)}=1.746$, p=n.s.), but interestingly, there was a significantly higher number of immune cell aggregates in livers of LE-sucrose rats compared to LE-grain rats ($F_{feeding\ regime(1,24)}= 5.52$, $p<0.05$).

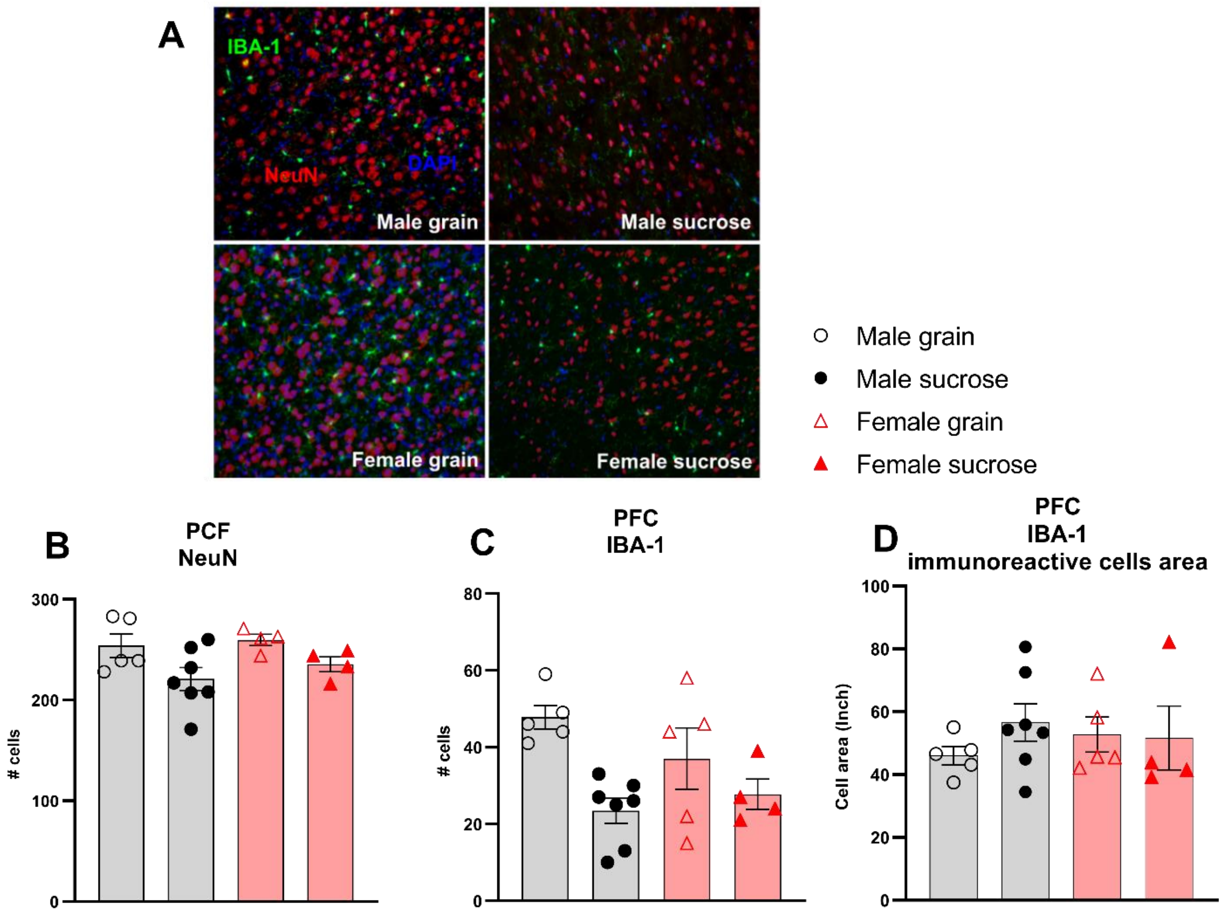

Figure S6. Number of NeuN, IBA-1 positive cells and IBA-1 immunoreactive cells area in the medial prefrontal cortex (PFC) of male and female LE-sucrose and LE-grain rats. (A) Representative images of NeuN and IBA-1 immunoreactive cells. (B) Number of NeuN, (C) Number of IBA-1 cells and (D) immunoreactive cell area of representative IBA-1 cells. Values represent the group mean (±SEM). There was a significant decrease the number of NeuN ($F_{feeding\ regime(1,16)}$= 6.75, $p<0.05$) and IBA-1 cells ($F_{feeding\ regime(1,17)}$=0.44, $p<0.01$) in LE-sucrose rats compared to LE-grain rats, but without sex effects. No significant effects of feeding regime and sex was found for the IBA-1 immunoreactive cells area ($F_{genotype}(1,17)$=0.5458, p=n.s.; $F_{sex}(1,17)$=0.01982, p=n.s.).

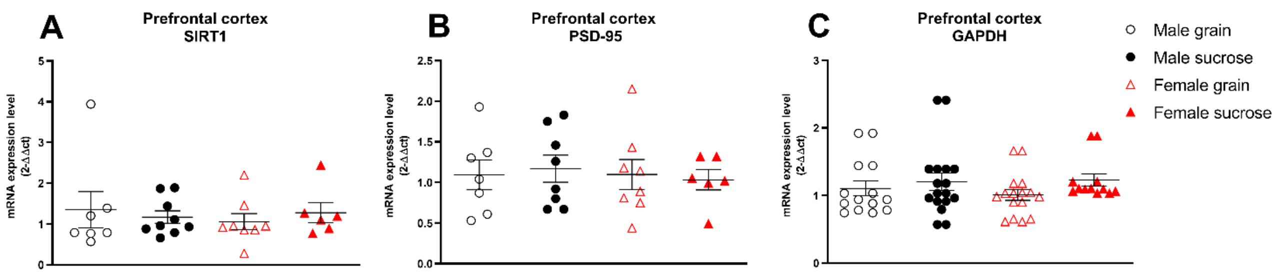

Figure S7. mRNA expression levels of SIRT1 (A), PSD-95 (B) and GADPH (C) in the medial prefrontal cortex of male and female LE-sucrose and LE-grain rats. Values represent the group mean (±SEM). No significant effect of feeding regime was observed (SIRT1: $F_{feeding\ regime(1,26)}$=0.004, p=n.s., $F_{sex(1,30)}$=0.1162, p=n.s.; PSD-95: $F_{feeding\ regime(1,25)}$=0.0008, p=n.s., $F_{sex(1,25)}$=0.1464, p=n.s.; GADPH: $F_{feeding\ regime(1,26)}$=0.9050, p=n.s., $F_{sex(1,26)}$=0.0282, p=n.s.).

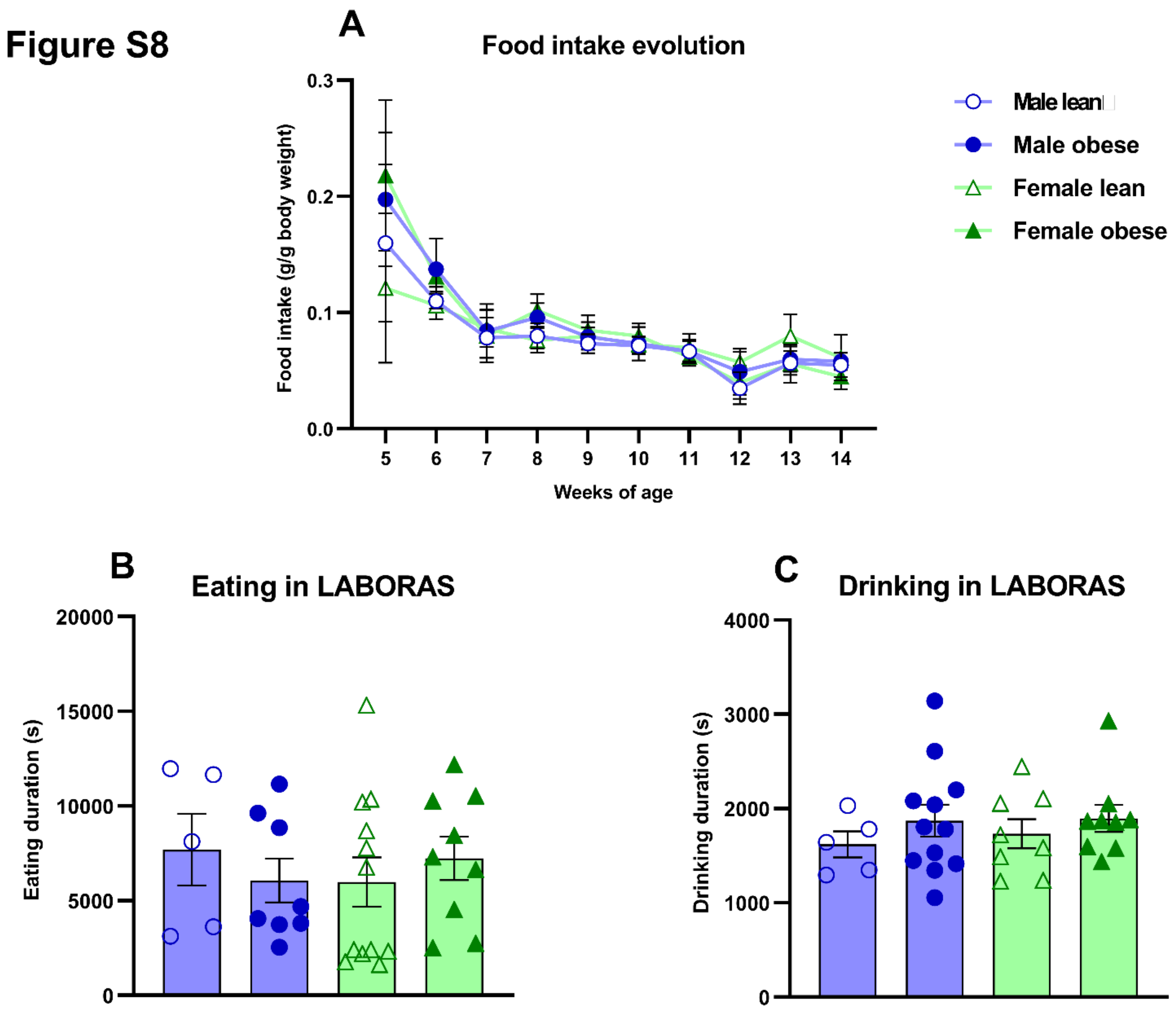

Figure S8. Food intake in male and female ZDF obese and lean rats. (A) Average of weekly food intake *ad libitum*. (B, C) Eating and Drinking duration (seconds) in Laboras homecages (Values represent the group mean of 5 sessions of 48 hours each in the Laboras homecage ± SEM).

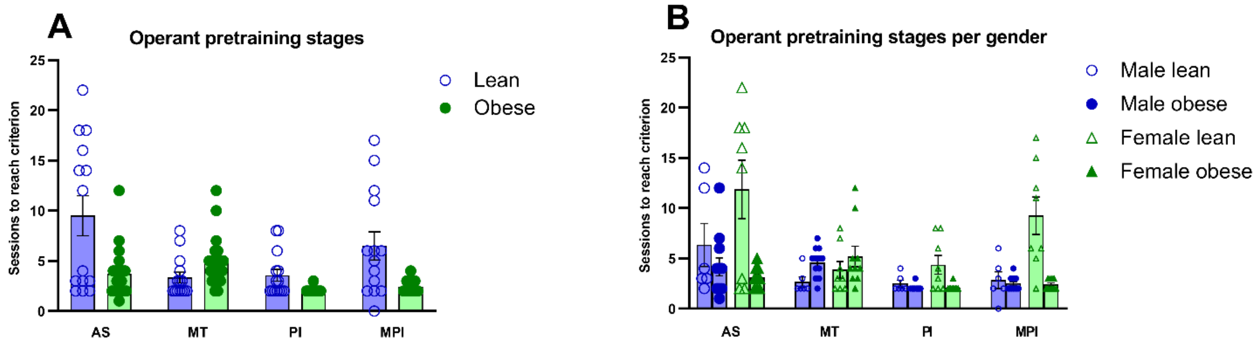

Figure S9. Number of sessions required for male and female obese and lean ZDF rats to reach criterion during the pretraining stages. (A) Number of sessions required to reach criterion during the pretraining stages for lean and obese rats of both sexes together. (B) Number of sessions required to reach criterion during the pretraining stages, specified per gender and genotype. Compared to males, females needed more session to reach criteria on punish incorrect and moving punish incorrect. Values represent the group mean (±SEM). AS = autoshaping; MT = must touch; PI = punish incorrect; MPI = moving punish incorrect.