Supplementary Results: Comparison of pair-fed and control groups

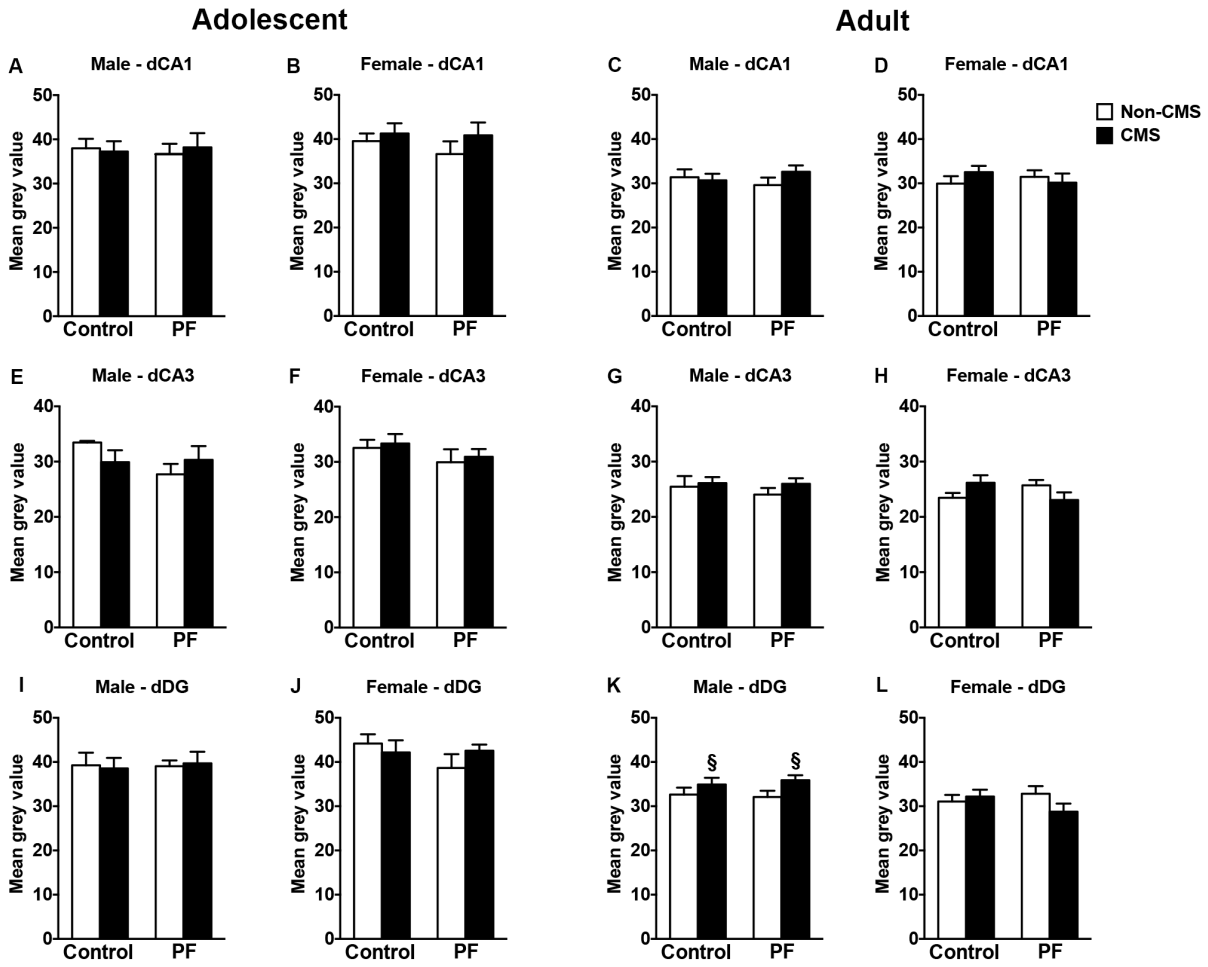
Mineralocorticoid mRNA expression

Dorsal hippocampal formation: In adolescence, neither pair-feeding nor adolescent CMS affected MR mRNA expression in the dorsal hippocampal formation of male and female rats (Supplementary Figure 1A-B, E-F, I-J). By contrast, adolescent CMS increased MR mRNA expression in the dorsal DG of adult males, independently of prenatal treatment [Supplementary Figure 1K; significant main effect of CMS for dorsal DG ($F_{(1,28)}$=4.58, p=0.04)]. Neither pair-feeding nor adolescent CMS affected MR mRNA expression in the dorsal hippocampal formation of adult females or in dorsal CA1 and CA3 of adult males (Supplementary Figure 1C-D, G-H, L).

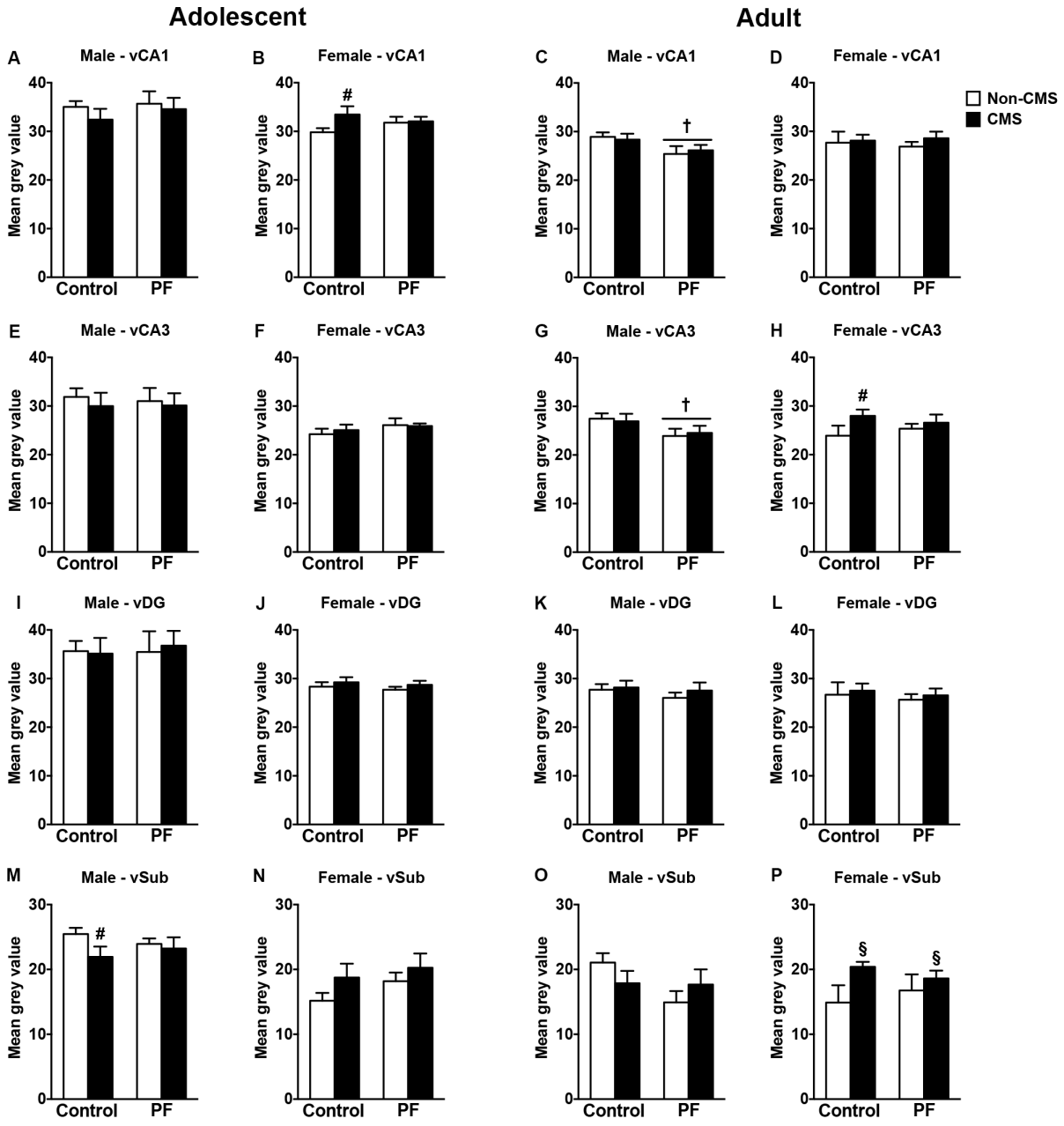
Ventral hippocampal formation: In adolescent males, CMS decreased MR mRNA expression in the ventral subiculum in control but not pair-fed rats, with no changes observed in the CA1, CA3, and DG [Supplementary Figure 2A,E,I,M; *a priori* analysis for ventral subiculum comparing control non-CMS to control CMS (p=0.04)]. In addition, CMS increased MR mRNA expression in the CA1 of control but not pair-fed adolescent females, [Supplementary Figure 2B; *a priori* analysis for CA1 comparing control non-CMS to control CMS (p=0.04)]. Neither pair-feeding nor adolescent CMS affected MR mRNA expression in the ventral CA3 and DG and subiculum of adolescent females (Supplementary Figure 2F,J,N).

In adulthood, MR mRNA expression was reduced in pair-fed males in the ventral CA1 and CA3, independently of adolescent CMS exposure [Supplementary Figure 2C,G; significant main effect of prenatal treatment for CA1 ($F_{(1,28)}$=5.28, p=0.03) and CA3 ($F_{(1,28)}$=4.47, p=0.04)]. However, neither pair-feeding nor CMS affected MR mRNA expression in the ventral DG and subiculum of adult males (Supplementary Figure 2K,O). By contrast, adolescent CMS increased

MR mRNA expression in the CA3 of control but not pair-fed females and increased MR mRNA expression in the ventral subiculum of both control and pair-fed females [Supplementary Figure 2H,P; *a priori* analysis comparing control non-CMS to control CMS for CA3 (p=0.05); significant main effect of CMS for ventral subiculum ($F_{(1,28)}$=4.22, p=0.049)]. Neither pair-feeding nor CMS affected MR mRNA expression in the ventral CA1 and DG of adult females (Supplementary Figure 2D,L).



Supplementary Figure 1. Short- and long-term effects of adolescent CMS on dorsal hippocampus MR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of MR mRNA expression in the CA1 (A-D), CA3 (E-H), and DG (I-L). § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS (n = 6-10 for all groups).



Supplementary Figure 2. Short- and long-term effects of adolescent CMS on ventral hippocampus MR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of MR mRNA expression in the CA1 (A-D), CA3 (E-H), DG (I-L), and ventral subiculum (M-P). † indicates a significant main effect of prenatal treatment, where all pair-fed animals are different from control animals; § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS; for B, H, and M, # indicates that control CMS is different from control non-CMS based on *a priori* comparisons (n = 4-10 for all groups).

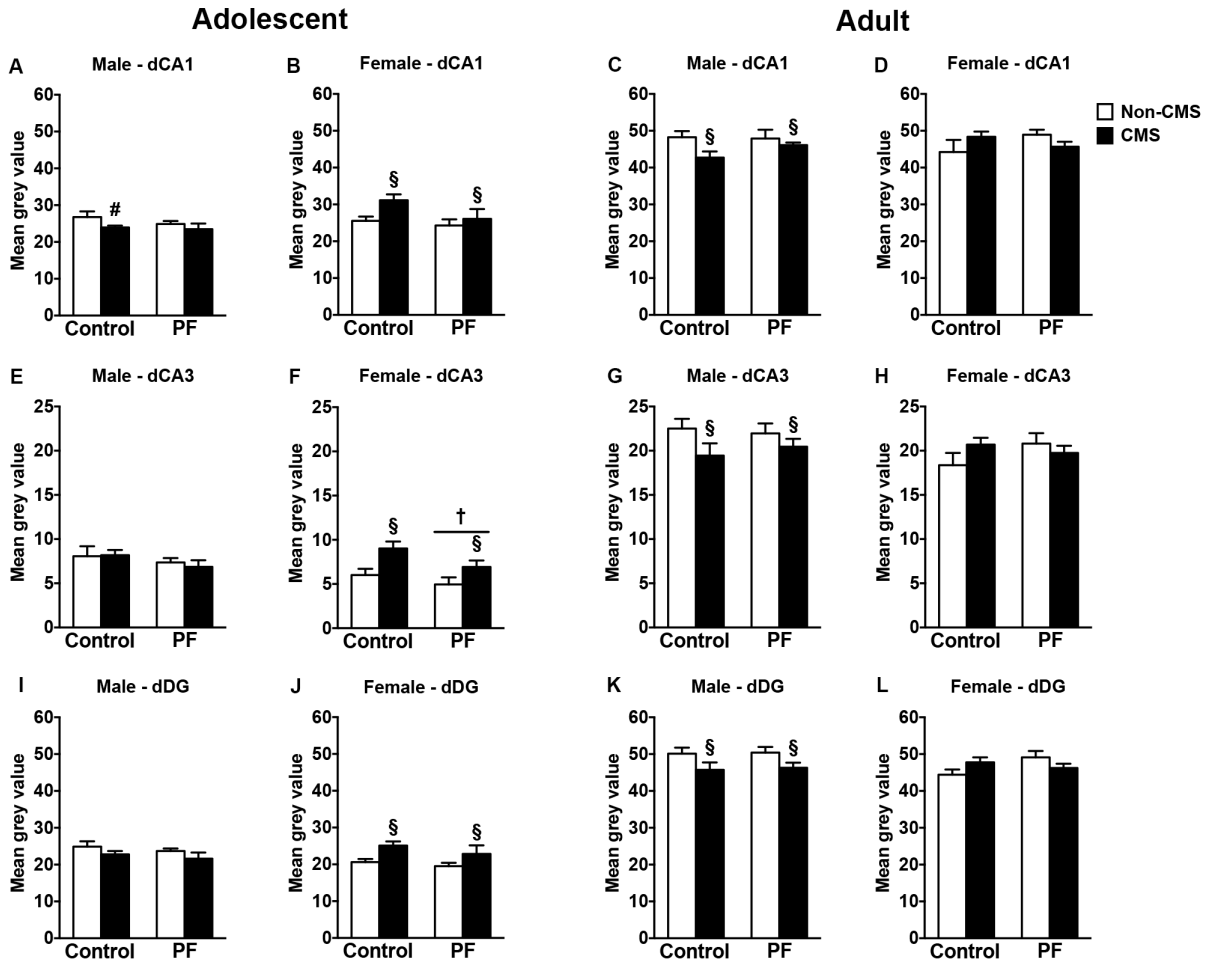
Glucocorticoid mRNA expression

Dorsal hippocampal formation: In adolescent males, CMS decreased GR mRNA expression in CA1 in control but not pair-fed rats, but no changes were observed in CA3 and DG [Supplementary Figure 3A,E,I; *a priori* analysis for CA1 comparing control non-CMS to control CMS (p=0.05)]. In adolescent females, however, CMS increased GR mRNA expression in CA1, CA3, and DG in both control and pair-fed animals, independently of prenatal exposure (Supplementary Figure 3B,F,J). Pair-feeding also reduced GR mRNA expression in CA3, independently of CMS exposure [significant main effects of CMS for CA1 ($F_{(1,27)}$=4.28, p=0.048), CA3 ($F_{(1,28)}$=10.76, p=0.003), and DG ($F_{(1,28)}$=7.56, p=0.01) and significant main effect of prenatal treatment for CA3 ($F_{(1,28)}$=4.34, p=0.046)].

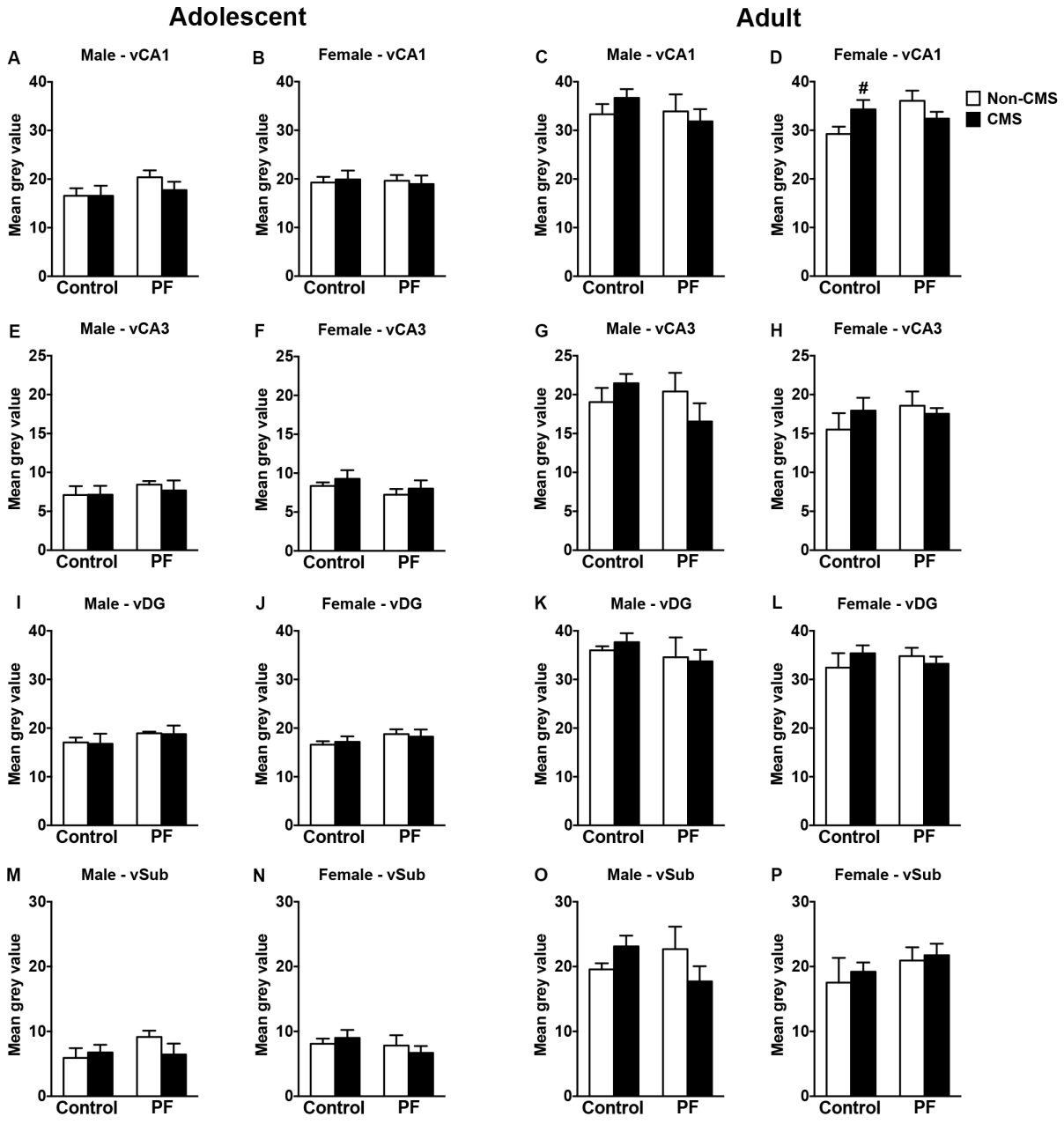
In adult males, CMS during adolescence reduced GR mRNA expression in CA1, CA3, and DG in both control and pair-fed animals [Supplementary Figure 3C,G,K; significant main effects of CMS for CA1 ($F_{(1,27)}$=4.26, p=0.05), CA3 ($F_{(1,28)}$=4.02, p=0.05), and DG ($F_{(1,28)}$=6.50, p=0.02)]. Neither pair-feeding nor adolescent CMS affected GR mRNA expression in the dorsal CA1, CA3, and DG of adult females (Supplementary Figure 3D,H,L).

Ventral hippocampal formation: Neither pair-feeding nor adolescent CMS affected GR mRNA expression in the ventral hippocampal formation of adolescent males and females (Supplementary Figure 4A-B,E-F,I-J,M-N).

In adulthood, neither pair-feeding nor adolescent CMS affected GR mRNA expression in the ventral CA1, CA3, DG or subiculum of males (Supplementary Figure 4C,G,K,O). In adult females, adolescent CMS increased GR mRNA expression in the CA1 only in controls, but no changes were observed in CA3, DG, and ventral subiculum [Supplementary Figure 4D,H,L,P; *a priori* analysis for CA1 comparing control non-CMS to control CMS (p=0.04)].



Supplementary Figure 3. Short- and long-term effects of adolescent CMS on dorsal hippocampus GR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of GR mRNA expression in the CA1 (A-D), CA3 (E-H), and DG (I-L). † indicates a significant main effect of prenatal treatment, where all pair-fed animals are different from control animals; § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS; for A, # indicates that control CMS is different from control non-CMS based on *a priori* comparisons (n = 6-10 for all groups).



Supplementary Figure 4. Short- and long-term effects of adolescent CMS on ventral hippocampus GR mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of GR mRNA expression in the CA1 (A-D), CA3 (E-H), DG (I-L), and ventral subiculum (M-P). For D, # indicates that control CMS is different from control non-CMS based on *a priori* comparisons; (n = 5-10 for all groups).

CRHR1 mRNA expression

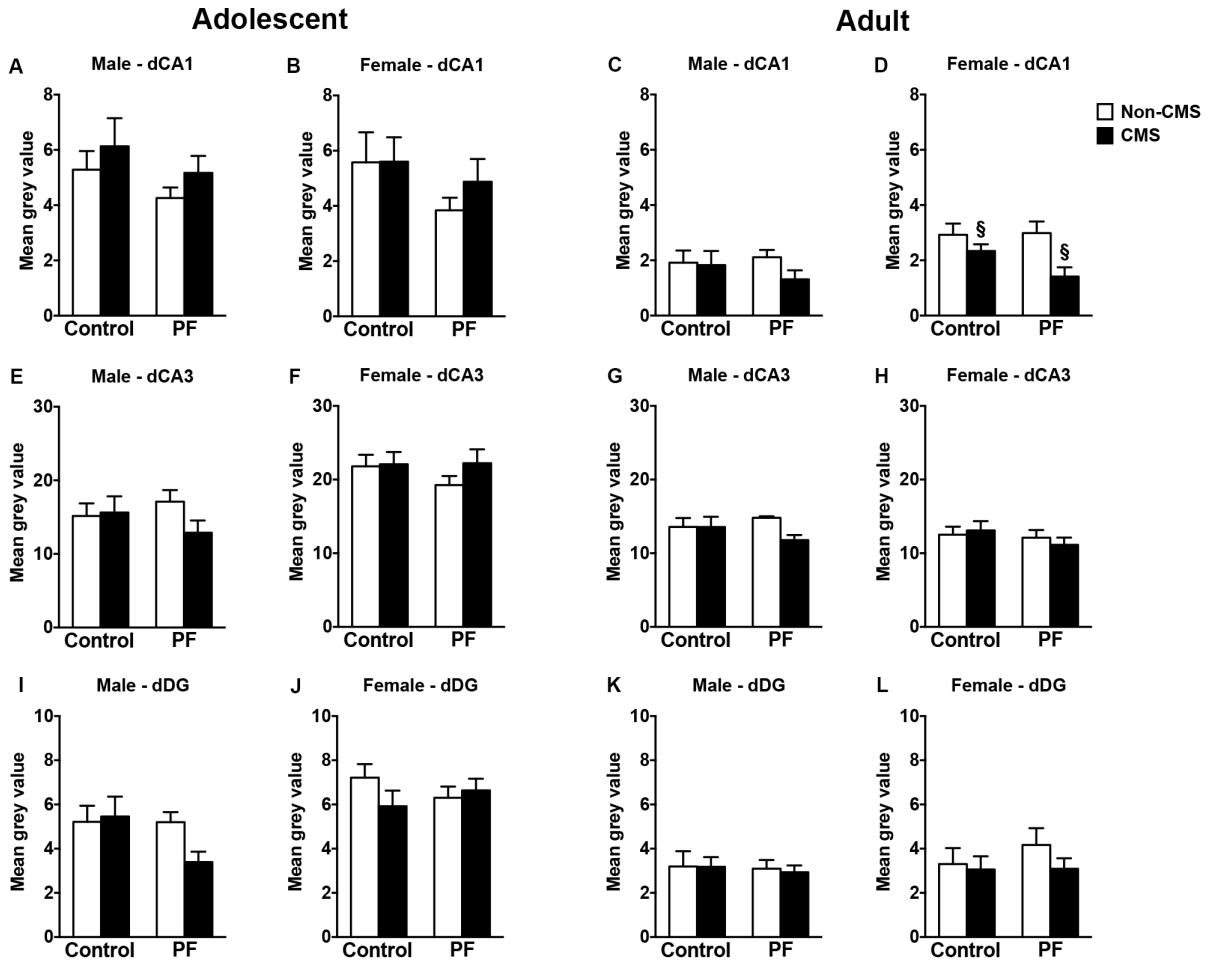
Dorsal hippocampal formation: Neither pair-feeding nor adolescent CMS affected CRHR1 mRNA expression in the dorsal hippocampal formation of adolescent males or females (Supplementary Figure 5A-B,E-F,I-J).

In adult males, neither pair-feeding nor adolescent CMS affected CRHR1 mRNA expression in the dorsal hippocampal formation (Supplementary Figure 5C,G,K). In adult females, however, adolescent CMS decreased CRHR1 mRNA expression in CA1 in both control and pair-fed animals, independently of prenatal exposure, but no changes were observed in the CRHR1 mRNA expression in the CA3 and DG [Supplementary Figure 5D,H,L; significant main effect of CMS for CA1 ($F_{(1,28)}$=9.61, p=0.004)].

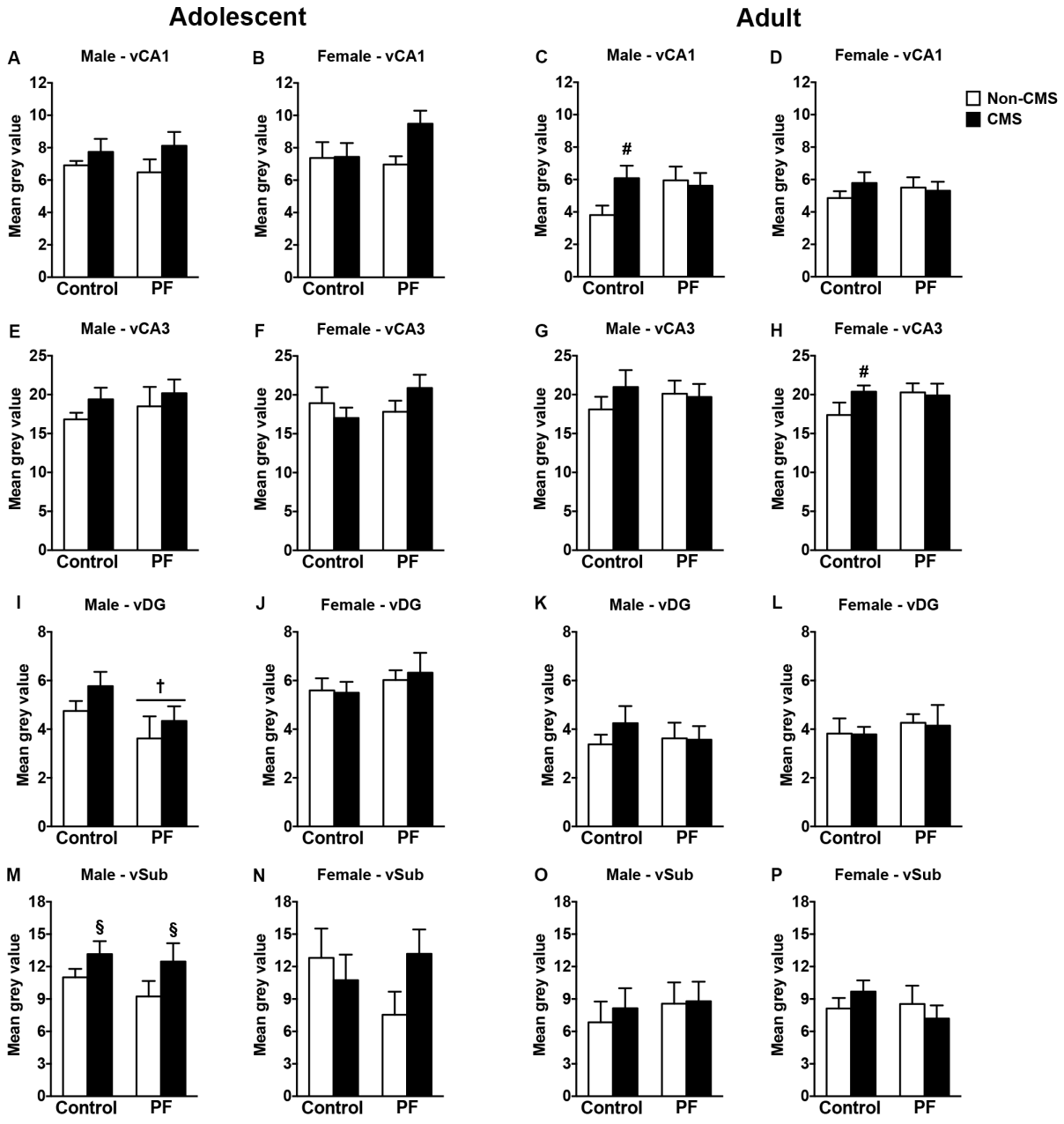
Ventral hippocampal formation: In adolescent males, pair-feeding decreased CRHR1 mRNA expression in the DG, independently of CMS exposure [Supplementary Figure 6I; significant main effect of prenatal treatment for DG ($F_{(1,25)}$=4.27, p=0.05)]. Moreover, CMS increased CRHR1 mRNA expression in the subiculum in both control and pair-fed males, independently of prenatal exposure [Supplementary Figure 6M; significant main effect of CMS for subiculum ($F_{(1,22)}$=4.51, p=0.04)]. Neither pair-feeding nor CMS affected CRHR1 mRNA expression in the CA1 and CA3 of adolescent males (Supplementary Figure 6A,E). In adolescent females, neither pair-feeding nor CMS affected CRHR1 mRNA expression in the ventral hippocampal formation (Supplementary Figure 6B,F,J,N).

In adult males, CMS during adolescence increased CRHR1 mRNA expression in the CA1 of control but not pair-fed animals [Supplementary Figure 6C; *a priori* analysis for CA1 comparing control non-CMS to control CMS (p=0.02)]. Neither pair-feeding nor CMS affected CRHR1 mRNA expression in the CA3, DG, and ventral subiculum of adult males

(Supplementary Figure 6G,K,O). In adult females, adolescent CMS increased CRHR1 mRNA expression in the CA3 of control animals [Supplementary Figure 6H; *a priori* analysis for CA3 comparing control non-CMS to control CMS (p=0.04)]. Neither pair-feeding nor CMS affected CRHR1 mRNA expression in the CA1, DG, and ventral subiculum of adult females (Supplementary Figure 6D,L,P).



Supplementary Figure 5. Short- and long-term effects of adolescent CMS on dorsal hippocampus CRHR1 mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of CRHR1 mRNA expression in the CA1 (A-D), CA3 (E-H), and DG (I-L). § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS (n = 6-10 for all groups).



Supplementary Figure 6. Short- and long-term effects of adolescent CMS on ventral hippocampus CRHR1 mRNA expression in control and pair-fed (PF) rats. Bars represent the mean ± SEM (mean gray value) of CRHR1 mRNA expression in the CA1 (A-D), CA3 (E-H), DG (I-L), and ventral subiculum (M-P). † indicates a significant main effect of prenatal treatment, where all pair-fed animals are different from control animals; § indicates a significant main effect of CMS exposure, where all animals exposed to CMS are different from animals not exposed to CMS; for C and H, # indicates that control CMS is different from control non-CMS based on *a priori* comparisons (n = 5-10 for all groups).

Supplementary Discussion

Pair-feeding

The pair-fed group is often used as a secondary control group in animal studies exploring the effects of maternal alcohol consumption on offspring. The pair-fed group was initially included to control for the decreased food intake that is typically observed with chronic alcohol consumption, as an attempt to separate the alcohol effects from those of possible undernutrition, and inclusion of a pair-fed group has become a customary procedure. However, this group is at best an imperfect and/or confounded control and at worst, an experimental treatment in itself. Indeed, pair-feeding can only control for the *reduced food intake* of the alcohol-consuming animals, but can never account for any of the nutritional effects associated with alcohol consumption, including alterations in absorption and utilization of nutrients (Weinberg, 1984) and increases in satiety (Lin et al., 1998). Furthermore, because pair-fed animals receive a reduced food portion – less than they would consume if allowed to eat *ad libitum* – they generally consume their entire day's ration within a few hours, and are thus essentially food deprived until their next feeding (Gallo and Weinberg, 1981; Weinberg 1984). In addition to inducing an abnormal feeding pattern, the pair-feeding procedure also introduces a mild prenatal stress component (the pregnant female is hungry for much of the day), which in itself may have long-term impacts on offspring developing neurobiological systems, including the neuroendocrine axis (Vieau et al., 2007).

Pair-feeding and expression of stress-related receptors in the hippocampus

In the present study, pair-feeding resulted in unique changes in the expression of stress-related receptors in the hippocampus. Importantly, none of these pair-fed effects are shared with

the PAE effects. Indeed, different from PAE, pair-feeding uniquely reduced MR expression in the ventral CA1 and CA3 of adult males, reduced GR expression in dorsal CA3 of adolescent females, and reduced CRHR1 expression in ventral DG of adolescent males. Conversely, there are several instances where PAE affected the expression of stress-related receptors in the hippocampus and pair-feeding had no effects. Indeed, different from pair-feeding, PAE uniquely reduced MR expression in dorsal CA1, CA3, and DG of adolescent males, increased MR in the ventral subiculum of adolescent females, reduced GR in the dorsal CA1 and DG and increased GR in ventral CA1 of adolescent females; reduced CRHR1 in dorsal CA1 of adolescent males; reduced CRHR1 in ventral DG of adolescent females, and reduced CRHR1 in dorsal CA1 and DG of adult females.

In summary, the current findings suggest unique effects of pair-feeding and provide further evidence that pair-feeding is not an ideal control group for models evaluating the effects of alcohol consumption during pregnancy. The effects observed in pair-fed offspring could, in themselves, lead to negative long-term effects for the neuroendocrine systems that are quantitatively and qualitatively different from those changes observed in PAE offspring.

Supplementary References

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